Phytoplankton as a principal diet for callianassid shrimp larvae in coastal waters, estimated from laboratory rearing and stable isotope analysis

Yu Umezawa[1,2,*], Akio Tamaki[1], Toshikazu Suzuki[1], Seiji Takeuchi[1], Chikage Yoshimizu[3], Ichiro Tayasu[3]

[1]Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo-machi, Nagasaki 852-8521, Japan

[2]Department of Environmental Science on Biosphere, Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

[3]Research Institute for Humanity and Nature, Motoyama, Kamigamo, Kyoto 603-8047, Japan

*Corresponding author: umezawa@me.tuat.ac.jp

ABSTRACT: The field diet of meroplanktonic decapod crustacean larvae is poorly known, despite standard use of microzooplankton as food in laboratory culture. Using callianassid shrimp *Nihonotrypaea harmandi* larvae collected from a 65 m deep inner-shelf location off mid-western Kyushu, Japan, between June and August 2012 and 2013 and mass-reared in the laboratory, phytoplankton-based diet through larval development (zoeae I–VI to decapodid) was demonstrated. When the pure-cultured diatom *Chaetoceros gracilis* was fed to zoeae, survival rate to decapodids was 3.4 to 3.9% in 26 to 40 d at 22°C, which was comparable to previous rearing results for zoeae fed microzooplankton. Trophic enrichment factors (TEFs)

from stable isotope (SI) analysis of zoeal whole-body tissue in the laboratory were 2.0‰ for $\delta^{13}C$ and 1.9‰ for $\delta^{15}N$. In the field water column, diatoms dominated the nano- to micro-sized plankton, accounting for 38 to 81% of the biovolume, followed by heterotrophic protists. The trophic position (TP) estimated from amino acid-specific $\delta^{15}N$ values for the field-collected zoeae VI was 2.1 ($TP_{Glu/Phe}$) or 2.7 ($TP_{Ala/Phe}$), suggesting that those zoeae fed on mixtures of phytoplankton and heterotrophs including protists. Bulk SI analyses were performed for particulate organic matter (POM; proxy for phytoplankton), microzooplankton (mainly calanoid copepods), and shrimp zoeae to elucidate the diet of larvae in the water column. A shift in SI from fresh to degraded POM was determined through the incubation of field-collected POM. Based on this shift during degradation and larval TEFs, phytoplankton and their sinking detritus with heterotrophic protists were estimated to be the principal diet for those larvae residing mostly below the chlorophyll maximum layer.

KEY WORDS: *Nihonotrypaea harmandi* · Zoea · Rearing · Diatom · Isotopic trophic enrichment factor · Amino-acid-$\delta^{15}N$-based trophic position · Phytodetritus · Protists

INTRODUCTION

Meroplanktonic larvae of macrobenthos are a seasonally significant component of zooplankton assemblages in tropical, temperate, and boreal estuarine and coastal waters. The successful acquisition of food by planktotrophic larvae is one critical process in their normal development and survival (Thorson 1950, Day & McEdward 1984, Olson & Olson 1989, Metaxas & Saunders 2009). Decapod crustaceans are a common component of both benthic macro-invertebrate communities and meroplanktonic assemblages in these nearshore waters. The potential food sources for planktotrophic decapod larvae comprise bacteria, phytoplankton ranging from pico- to micro-sized groups, zooplankton ranging from nano- to meso-sized groups, and detritus and fecal pellets (Lebour 1922, Stickney & Perkins 1981, Factor & Dexter 1993, Mascetti & Wehrtmann 1996, Lehto et al. 1998, Anger 2001, Schwamborn et al. 2006,

49 Fileman et al. 2014). In laboratory rearing of decapod larvae, brine shrimp *Artemia* spp. nauplii
50 and rotifers (e.g. *Brachionus* spp.), which are microzooplankton never encountered by those
51 larvae in their natural environment, have most frequently been used for complete larval
52 development from newly hatched to post-larval stages (McConaugha 1985, Harvey & Epifanio
53 1997, Anger 2001, Calado et al. 2010). Such success in larval culture, often with excess supply
54 of those food items, does not necessarily mean that microzooplankton are a principal food
55 source for natural larval populations, as individual density, microscale patchiness, filterability
56 (e.g. non-motile phytoplankton by filter-feeding larvae) or catchability (e.g. motile
57 phytoplankton or zooplankton by raptorial larvae), digestibility, and nutritional value are likely
58 to play their roles in natural diets. Principal diets of decapod crustacean larvae under natural
59 conditions thus remain to be determined.

60 Successful rearing of planktotrophic larvae of species in 1 of the 2 decapod suborders,
61 Pleocyemata, to the decapodid (= post-larval) or juvenile stage solely using phytoplankton has
62 been achieved in only a few studies on brachyuran crabs (Atkins 1955, Bousquette 1980,
63 Harms & Seeger 1989). In the latter study, larvae of a majid fed a diatom species showed a
64 longer developmental duration and lower survival rate than those fed *Artemia* sp. nauplii. In
65 other studies, phytoplankton are regarded as partially valid foods, typically compared with 2
66 control rearing treatments, either fed *Artemia* spp. nauplii (complete) or starved (invalid)
67 (Table 1). For zoeal stages in penaeid shrimp (suborder Dendrobranchiata), phytoplankton
68 (mainly diatoms) are considered a fully effective food source (Preston et al. 1992, Yano 2005).

69 Even if phytoplankton are a principal diet of meroplanktonic decapod crustacean
70 larvae to complete their development in the field, examination alone of ingested food species
71 contained in larval digestive tracts or fecal pellets, including algal cell walls, photosynthetic
72 pigments, and DNA-identified amorphous material, will not suffice to reach a convincing
73 conclusion. In addition to live forms, phytodetritus can be a potential food source for decapod

74 larvae (Meyer-Harms & Harms 1993, Kiørboe 2011, Turner 2015). Although carbon and
75 nitrogen stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis is one promising way to estimate diets of those
76 larvae, the number of actual isotope studies is scarce. In a study area ranging from a mangrove
77 shore to an offshore shelf in northeastern Brazil, the contribution of mangrove carbon to
78 decapod larval nutrition was regarded as negligible, and pelagic primary producers were
79 suggested as principal diet sources (Schwamborn et al. 1999, 2002). However, the estimation
80 of the trophic position (TP) and food sources for target species based on such 'bulk' isotope
81 analysis has uncertainties. First, it is impossible to cover all potential food sources in the field,
82 where a variety of primary producers and consumers exist. Second, isotope values of primary
83 producers are variable in space and time due to the variability of their species composition, and
84 dissolved inorganic carbon and nitrogen sources. Finally, as enrichment of ^{13}C and ^{15}N in
85 biological tissues across trophic levels is variable depending on the species involved,
86 commonly used trophic enrichment factors (TEFs) (ca. 1‰ for ^{13}C and 3 to 4‰ for ^{15}N; e.g.
87 DeNiro & Epstein 1978, Minagawa & Wada 1984) in place of species-specific ones (e.g.
88 Yokoyama et al. 2005) may mislead the interpretation of food-chain structure. In addition to
89 bulk analysis, $\delta^{15}N$ of amino acids (AAs) have been widely used to estimate more accurate TPs
90 (McClelland & Montoya 2002, Ohkouchi et al. 2017 and references therein). Among the AAs
91 found in organisms, 2 types have been identified in terms of their ^{15}N-enrichment across
92 trophic levels. One is 'trophic amino acids', the $\delta^{15}N$ of which significantly increase from one
93 trophic level to the next, while the other, 'source amino acids', show only slight shifts in $\delta^{15}N$
94 between trophic levels. Therefore, the analysis of $\delta^{15}N$ in a set of AAs covering these 2 types
95 (mainly phenylalanine for source AA and glutamic acid for trophic AA) for a target species
96 potentially enables the identification of accurate TPs (Chikaraishi et al. 2009). Furthermore, it
97 has recently been reported that one trophic AA, alanine, is effective at more correctly
98 estimating a trophic pathway including both protistan and metazoan consumers
99 (Gutiérrez-Rodríguez et al. 2014, Décima et al. 2017, Landry & Décima 2017). Therefore, the

100 use of alanine as trophic AA would be more adequate for food chain analyses in coastal oceans
101 as well as pelagic waters where trophic transfers through protistan grazers are not negligible.

102 Callianassid shrimp or ghost shrimp (Pleocyemata: Axiidea) play key roles as
103 ecosystem engineers and community organizers in estuarine and coastal marine sedimentary
104 habitats (Pillay & Branch 2011, Dworschak et al. 2012). Planktotrophic larvae of callianassids
105 go through 3 to 7 zoeal stages to reach the decapodid stage (Kubo et al. 2006, Pohle et al. 2011,
106 Kornienko et al. 2015). The survival rate of those larvae significantly affects the population
107 dynamics of adults, which could further exert an impact on the community and ecosystem
108 surrounding that population. Following the release of zoeae I from the shore, those larvae grow
109 in the inner-shelf coastal ocean (Johnson & Gonor 1982, Yannicelli et al. 2006, Tamaki et al.
110 2010).

111 In midwestern Kyushu, Japan, a population of the callianassid shrimp *Nihonotrypaea*
112 *harmandi* (Bouvier 1901) is distributed on intertidal sandflats in a region ranging from estuary
113 (Ariake Sound) to inner-shelf coastal ocean (Amakusa-nada) (Tamaki at al. 1999) (Fig. 1).
114 Around the western edge of the region, the largest local population in the region exists on the
115 intertidal sandflat in Tomioka Bay (Tomioka sandflat; 32.521° N, 130.037° E) (Tamaki &
116 Harada 2005, Tamaki & Takeuchi 2016). Tomioka Bay forms a coastal boundary layer with
117 water depths ≤30 m. Larvae released from the sandflat are transported across Tomioka Bay to
118 Amakusa-nada, where the main nursery ground for larvae lies 10 to 20 km north to west of the
119 sandflat (Tamaki & Miyabe 2000, Tamaki et al. 2010, 2013). Complete rearing of *N. harmandi*
120 larvae has been achieved in the laboratory using the rotifer *Brachionus rotundiformis*, and
121 newly hatched *Artemia* sp. nauplius (Konishi et al. 1999). By contrast, examination of both the
122 mouthpart morphology and digestive tract content of field-caught *N. harmandi* larvae suggests
123 that they are herbivorous, mainly feeding on diatoms (Somiya et al. 2014). But there are no
124 records of complete larval rearing solely with phytoplankton.

125 The objective of the present study was to test whether phytoplankton and/or
126 phytoplankton-derived detritus (phytodetritus) are the principal food source for zoeal larvae of
127 *N. harmandi* in the coastal ocean. First, larvae of *N. harmandi* were mass-reared with
128 pure-cultured phytoplankton as a single food source in the laboratory. Second, the taxonomic
129 composition of nano- and micro-plankton over the water column in the field was determined to
130 support the analysis of food sources for the zoeal larvae. Finally, stable isotope values of bulk
131 and individual AAs were determined for field samples to estimate the actual food source. The
132 TEFs for the bulk $\delta^{13}C$ and $\delta^{15}N$ values in zoeal larvae were determined for a sample from the
133 above rearing experiment. The shift in $\delta^{13}C$ and $\delta^{15}N$ values associated with microbial
134 degradation of phytoplankton was obtained by a laboratory incubation of particulate organic
135 matter in order to incorporate phytodetritus into the food-chain diagram as a potential food
136 source. It was concluded that mixtures of phytoplankton and heterotrophs including protists
137 were the most likely diet for field larvae of *N. harmandi*.

138 # MATERIALS AND METHODS

139 ## Biology of ghost shrimp adults and larvae

140 Using the TEFs for juveniles of *Nihonotrypaea harmandi* reared with pure-cultured
141 diatoms *Chaetoceros gracilis* in the laboratory (Yokoyama et al. 2005), the diet of adults
142 collected from the Tomioka sandflat was estimated to be phytoplankton and benthic
143 microalgae (Shimoda et al. 2007). The reproductive season for the *N. harmandi* population
144 spans June through October. Larval development consists of 6 zoeal (up to the zoea VI) and 1
145 decapodid stages (Konishi et al. 1999, Somiya et al. 2014). Maximum total length for zoeae I
146 and VI is ca. 3 and 7 mm, respectively. Within the water column at a 65 m deep location 10 km
147 west of the sandflat in Amakusa-nada (Stn A in Fig. 1; 32.545° N, 129.942° E), the mean
148 vertical positions of most zoeal stages were at 30 to 40 m depths with prevailing temperatures

of 21 to 22°C and salinities of 33.5 to 34 during the end of July to early August 2006 (Tamaki et al. 2010). In the laboratory at 21°C, it took 30 d for most of the newly hatched zoeae reared with *Brachionus rotundiformis*, and newly hatched *Artemia* sp. nauplii to reach the decapodid stage (Tamaki et al. 2013). Starved zoeae I survived for up to 5 d and never proceeded to the zoea II stage (Y. Saitoh & A. Tamaki unpubl. data).

Setup for ghost shrimp larval rearing in the laboratory

Larvae of *N. harmandi* were mass-reared in 2 black, semi-cylindrical polyethylene tanks (rearing tanks 1 and 2) with a 30 l capacity during July and August 2013. Surface water from a coastal ocean area, filtered through a 10 µm mesh (TOCEL; JFE Advantech), was stored and used for the rearing experiment. The water temperature in the tanks was kept at around 22°C (which corresponded to the field water-column conditions for the larvae) using 2 water baths (see Section 1.1 in the Supplement at www.int-res.com/articles/suppl/mXXXpXXX_supp.pdf). The seawater temperature and salinity in each tank were recorded every 1 min with a Compact-CT (JFE Advantech). Gentle aeration and slow water circulation in each tank was made with a stream of tiny air bubbles passed through 2 air stones with 20 mm φ, which kept only food particles (i.e. not deposits) suspended. During rearing, the room lights were kept off as a rule, except during water exchanges, food supply, and retrieval of larvae. Furthermore, the tank tops were covered with fine-mesh black sheets, which was intended to approximate faint daylight conditions at 30 to 40 m water depths in Amakusa-nada (Tamaki et al. 2010).

Collection of ghost shrimp ovigerous females and initiation of larval rearing

During daytime low tide on the Tomioka sandflat on 7 July 2013, 57 females of *N. harmandi* with well-developed embryos were collected. These embryos were expected to hatch during the immediate nighttime as determined by their large eyes and transparent bodies due to

173 a minimum amount of remaining yolks. In the laboratory, the females were kept in containers
174 with filtered seawater until their larvae were released. Synchronized mass-release events
175 occurred twice, at 02:20 and 04:00 h on 8 July. Each time, swimming larvae (newly hatched
176 zoeae I) were collected with a nylon net and gently transferred to a transparent container
177 containing 1.5 to 3.0 l filtered seawater. From each container, 12 samples of 5 ml water were
178 randomly taken with a measuring pipette. After counting the number of larvae in each sample,
179 they were returned to the container for re-sampling. The mean (±SD) numbers of larvae
180 sample^{-1} (12.0 ± 4.0 and 3.7 ± 1.2 in the first and second events, respectively) were used to
181 estimate the total numbers of larvae, which were approximately 7200 and 1100 larvae in the
182 first and second events, respectively. These 2 batches of larvae were mixed in a cup with
183 filtered seawater and split into 2 cups each with a known volume of water. It was estimated that
184 4500 and 3800 larvae were contained in these cups. In this order, the cup contents were gently
185 poured into rearing tanks 1 and 2, respectively, each of which contained a sufficient quantity of
186 pure-cultured diatoms (*C. gracilis*; Bivalve Culture Institute, Sasebo city). All the above
187 procedures were completed by 05:00 h. The live diatom stock (approximately 5×10^8 cells ml^{-1}
188 in a bottle of 1000 ml seawater) can be stored at between 0 and 5°C in the refrigerator for at
189 least 50 d (see Yokoyama et al. 2005 and Tamaki et al. 2013 for successfully rearing
190 decapodids and juveniles of *N. harmandi* with this product).

191 Routine procedures for ghost shrimp larval rearing and retrieval, and data
192 analysis

193 Starting from 09:00 h on 8 July 2013 (Day 0), the daily routine work for each of the 2
194 tanks was completed between 09:00 and 11:00 h. Carcasses, feces, and exuviae of *N. harmandi*
195 larvae, and *C. gracilis* flocs deposited on the tank bottom were removed with pipettes.
196 Three-fourths of the tank water was gently siphoned off, with a 63 µm mesh nylon net covering
197 the siphon entrance to prevent larvae from being inhaled, and replaced with new filtered

198 seawater stored in the room. A fixed number of new diatoms was spread over the tank water,
199 poured with a small quantity of filtered seawater in which the pre-dilution of concentrated
200 diatoms had been made. The daily ration of *C. gracilis* per ml in the tank water was
201 approximately 5.0 to 6.7 × 10^3 cells. These rations were determined by comparing the total
202 volume of diatoms, as a product of cell diameter (5 to 7 μm)-based sphere volume × total
203 number, with that of rotifers and/or brine shrimp nauplii, the supply of which provided in
204 excess to similar initial numbers of zoeae I had led to substantial decapodid yields (Tamaki et
205 al. 2013).

206 At some date intervals, to check whether the larval stages proceeded as in the previous
207 rearing experiments with rotifers and brine shrimp nauplii (Konishi et al. 1999, Tamaki et al.
208 2013), small numbers of larvae up to the zoea IV stage were collected with a pipette and fixed
209 with 5% neutralized seawater formalin during the above routine work (in total, 39 and 56
210 larvae from rearing tanks 1 and 2, respectively). No sampling for the zoeae V and VI was done
211 to obtain a maximum number of decapodids. All decapodids that emerged daily, which were
212 mostly moving horizontally close to the tank bottom, were collected with a pipette. They were
213 kept frozen at −20°C for later stable isotope (SI) analysis. On Day 40, all larvae including those
214 still at the zoeal stages were collected and fixed (decapodids) or stored for SI analysis (zoeae).

215 Usually, when rearing *N. harmandi* larvae, decapodids occur in 2 cohorts over time,
216 forming the corresponding normal-distribution groups in the daily emerging individuals (A.
217 Tamaki unpubl. data). Following Aizawa & Takiguchi (1999), with a modification to
218 Hasselblad (1966), such a composite normal-distribution group was separated into the
219 component distributions.

220 **Size-frequency distributions for ghost shrimp embryos and newly hatched**
221 **zoeae I**

222 The multiple cohorts of decapodids of *N. harmandi* occurring through the rearing dates
223 might reflect those cohorts that could exist already at the stages of newly fertilized embryos
224 and newly hatched zoeae I, with dimension differences between the cohorts. The subsequent
225 developmental durations can vary accordingly. To detect the presence of such initial cohorts,
226 females with (1) newly fertilized embryos and (2) embryos about to hatch were collected from
227 the Tomioka sandflat during daytime low tides on 24 and 26 August and 10 September 2014.
228 Determination of these embryonic states is detailed in Section 1.2 in the Supplement. The
229 females with embryos-(1) were individually fixed with 10% neutralized seawater formalin.
230 Those with embryos-(2) were brought alive to the laboratory.

231 Under a light microscope in the laboratory, the above embryonic-state-(1) was
232 confirmed as the stage before reaching the embryonic nauplius. In total, 73 females with those
233 embryos were obtained. Ten embryos were randomly removed from each female, and the
234 longer and shorter axes of the ellipse of each embryo in plane aspect were measured to 0.01
235 mm with an eyepiece micrometer attached to the microscope at 100× magnification. The mean
236 longer and shorter axis lengths were used to calculate the volume of a spheroid embryo with its
237 longer axis as the axis of revolution. Using data for all females combined, the embryonic
238 volume-frequency distribution was made. The females with embryonic-state-(2) were kept
239 individually in small containers with filtered seawater until their larval release during the
240 immediate nighttime. In total, 61 females released larvae, which were collected and fixed with
241 5% neutralized seawater formalin. For 10 randomly chosen larvae from each female, the
242 mid-dorsal total length in lateral aspect, from rostral spine to telson tips, was traced with a
243 camera lucida of a stereomicroscope at 30 to 50× magnification. The curve length was
244 measured to 0.01 mm using ImageJ 1.48v (http://imagej.nih.gov/ij/). Using the mean larval
245 total length from every female, a total-length-frequency distribution was made. Each
246 composite normal-distribution group was separated into the components as previously.

Sampling diatoms and larvae reared in the laboratory for SI analysis

The liquid with concentrated diatoms (*C. gracilis*) for the rearing of *N. harmandi* larvae was sampled for SI analysis on Days 0, 9, 15, and 28, and immediately processed (see Section 1.3 in the Supplement).

The numbers of *N. harmandi* larvae retrieved for SI analysis (combined from the 2 rearing tanks) consisted of approximately 50 to 60 for zoeae I immediately after hatching (retrieved separately from those larvae subsequently used for the rearing), 9 for zoeae IV on Day 15, 52 for decapodids (7 to 16 each on Days 28 and 30 to 33), and all zoeae remaining on Day 40 (20 zoeae V and 37 zoeae VI). After their defecation, these specimens were kept frozen at −20°C until analysis. The TEF associated with the larval feeding on diatoms was calculated as the difference in SI values between zoeal whole-body tissues and diatoms as follows, when the larval SI values became steady during the rearing: TEF ($\Delta\delta^{13}C$ or $\Delta\delta^{15}N$) = SI value (larvae) – SI value (diatoms).

Field sampling for SI and plankton composition analyses

Water and biological sampling for chemical analyses were carried out at Stn A in Amakusa-nada (Fig. 1) during 2 cruises in 2012 (10 to 12 July and 7 to 10 August) and 3 cruises in 2013 (27 to 29 June, 10 to 12 July, and 7 to 9 August) onboard the training vessel (T/V) 'Kakuyo-Maru' (Nagasaki University) equipped with 12 Niskin bottles (5 l) mounted on a rosette multiple sampler (RMS; General Oceanics) with the CTD probe (SBE 9/11; Seabird Electronics). Regarding the potential food sources for *N. harmandi* larvae, water samples for particulate organic matter (POM) to be used for SI analyses were collected, 2 or 3 times in each cruise, from the chlorophyll (chl) maximum layers and other 1 or 2 layers 5 to 10 m above or below each chl maximum layer (10 to 25 m depth except for August 2012 [30 to 50 m], as detected with the CTD probe), which was expected to contain high content of fresh

phytoplankton. Chl *a* concentrations in the water column (μg l^{-1}) were calculated from chl fluorescence at each layer, and conversion factor was obtained at Stn A during the shared cruises in August and September 2012 (S. Takeda pers. comm.). Each 5 to 10 l sample of seawater was filtered through a 200 μm nylon mesh and a pre-combusted filter (GF/F; 47 mm φ), and then the sample was gently washed with a few ml of distilled water. Samples were kept frozen at –20°C until analysis and processed in the same manner as for the diatom sample (see Section 1.3 in the Supplement).

Zoeae of *N. harmandi* (no decapodids were obtained) and their potential food sources, zooplankton (mostly composed of calanoid copepods), were collected for bulk SI analyses by vertical towing with a Norpac Net (0.45 m mouth diameter, 1.8 m length, and 330 μm mesh size) from 5 m above the seabed to the surface at Stn A at both low- and high-tide times. These plankton samples were immediately fixed with neutralized formalin solution (5% final conc.) and brought to the laboratory. Under a stereomicroscope, larval samples were separated into the 6 stages (zoea I to VI) both in 2012 and 2013, while zooplankton were collected only in 2012 and separated into 6 body-size fractions (3.5–2.5, 2.5–2.0, 2.0–1.5, 1.5–1.2, 1.2–0.8, and 0.8–0.3 mm). Prior to the SI analysis of these field-collected samples, the effect of preservation in formalin solution on the $\delta^{13}C$ and $\delta^{15}N$ values for zoeal tissues (i.e. difference in $\delta^{13}C$ and $\delta^{15}N$ values between the zoeal samples with and without formalin treatment) was tested for several zoeal stages and the values used for the calibration of the SI values for both zoeae and other zooplankton.

Zoeae of *N. harmandi* for $\delta^{15}N$ analysis in amino acids were collected on 10 August 2012 from 30 m depth by horizontally towing a ‘fish-larvae’ net (1.3 m mouth diameter, 4.5 m length, and 330 μm mesh size) at a speed of 1.5 knots for 10 min. The sample was immediately brought to the laboratory alive, and zoeae VI were selected under a stereomicroscope and kept frozen at –20°C until analysis. About a 12 h lag from sampling to freezing was enough for

296 those zoeae to evacuate their digestive tract contents, suggesting no contamination of zoeal
297 tissues with diet/gut contents.

298 Water samples for the taxonomic composition analysis of nano- to micro-sized
299 plankton were collected from 4 depth layers (0, 20, 40, and 60 m) with a bucket (0 m) or Niskin
300 bottles on 7 August 2012. Sampled water was immediately fixed with acid Lugol's solution
301 (2% in final conc.) and stored in cool and dark conditions. In the laboratory, 3.1 to 4.4 ml of
302 water sample was set in a Sedgewick–Rafter chamber (Guillard 1978) for each layer, so that
303 >300 of those plankton were included. All individuals larger than 4 μm in equivalent spherical
304 diameter (i.e. eukaryotic unicellular organisms and filamentous cyanobacteria) were observed
305 and identified to varying levels from class to suborder under an inverted biological microscope
306 equipped with a 60× objective lens (Olympus IX71). Plankton biovolume was individually
307 estimated assuming approximate geometrical figures such as spherical, ellipsoidal, cylindrical,
308 and conical shapes.

309 ## POM degradation experiment

310 To check for the potential alteration of $\delta^{13}C$ and $\delta^{15}N$ values in POM by microbial
311 degradation in the water column during suspension and sinking into deeper layers, laboratory
312 incubation experiments were conducted in June and July 2013. To prepare POM presumably
313 with a high content of phytoplankton, seawater samples collected from the chl maximum layer
314 at Stn A (preceding section) were filtered sequentially through 200 and 20 μm nylon meshes.
315 The organic matter trapped on the 20 μm nylon mesh was re-suspended in filtered seawater
316 (through GF/F) in duplicate 200 ml bottles. The sample bottles were immediately stored in a
317 portable incubator (CN-25C; Mitsubishi Electric) onboard and incubated at a temperature of
318 22°C in the dark for 1 wk. Duplicate POM samples were collected on pre-combusted GF/F
319 filters (25 mm φ) on 3 dates (Days 0, 2 or 3, and 5 or 7), and processed in the same manner as
320 for the diatom samples in the larval rearing (see Section 1.3 in the Supplement).

AA extraction and purification

Roughly powdered zoeal whole-body tissues of *N. harmandi* (zoea VI, 2 sets of about 20 ind., ca. 4 mg dry weight collected at Stn A in August 2012) were pre-treated prior to $\delta^{15}N$ analysis of individual AAs. The subsequent procedures followed the protocol given in Ishikawa et al. (2014), with some modifications (see Section 1.4 in the Supplement).

SI measurement of bulk and AA samples

For the zooplankton samples (including *N. harmandi* larvae) collected at Stn A in Amakusa-nada in 2012 and 2013, and from the rearing experiment for the larvae, approximately 0.3 to 0.5 mg of the sample in dry weight (i.e. 15 to 20 ind. for zoea I, 11 to 13 for zoea II, 9 to 11 for zoea III, 6 to 8 for zoea IV, 3 to 5 for zoea V, 3 each for zoea VI and decapodid, and 10 to 50 ind. for zooplankton depending on body-size groups, randomly selected from the sample stock) was transferred to silver capsules (SÄNTIS Analytical) and dried at 60°C after acidification with a few drops of 1 N HCI to remove inorganic carbon. The $\delta^{13}C$ and $\delta^{15}N$ values for diatoms (diet in the rearing experiment), POM (field sample and degradation experiment), and zooplankton samples were measured with an elemental analyzer and an isotope-ratio mass spectrometer (FLASH 2000-Conflo IV-Delta V Advantage; Thermo Fisher Scientific). Instrument precision was checked with a calibration standard (L-alanine) every 5 samples (standard deviation <0.12‰ for $\delta^{13}C$, <0.14‰ for $\delta^{15}N$). The $\delta^{15}N$ values for the samples with lower N contents (<20 μg) were eliminated from the results (e.g. N at POM degradation experiment), because the accuracy of the measured values can be low, despite the calibration with standards, due to a mass-dependent shift in $\delta^{15}N$ values in small-quantity samples (Ogawa et al. 2010).

The $\delta^{15}N$ values of the 3 kinds of AAs (alanine [Ala], glutamic acid [Glu], and phenylalanine [Phe]) were determined by gas chromatography/combustion/isotope ratio mass

spectrometry (GC/C/IRMS) using a Delta V plus isotope ratio mass spectrometer (Thermo Fisher Scientific) coupled to a gas chromatograph (GC7890A; Agilent Technologies) via a modified GC-Isolink interface consisting of combustion and reduction furnaces. The AA derivatives were injected into the GC column using a Gerstel PTV injector in solvent vent mode. The programs on temperature, retention time, and carrier gas flow rate in each process (i.e. injection, combustion, reduction, and separation in GC) followed the methods given in Ishikawa et al. (2014). The CO_2 generated in the combustion furnace was removed using a liquid N trap. Standard mixtures of 8 kinds of AAs ($\delta^{15}N$ ranging from –25.9 to +45.6‰) were analyzed with zoeal sample in turn to confirm the reproducibility of the isotope measurements. Standard deviations of the standards were better than 0.8‰ with a sample quantity of 5 nmol μl^{-1} N.

It was anticipated that protozoan consumers potentially contributed to the diet of *N. harmandi* larvae (cf. Fileman et al. 2014); there is a certain amount of biomass of protozoa such as ciliate and heterotrophic dinoflagellate in coastal waters, and sinking phytodetritus with heterotrophs could be a candidate for the diet. Therefore, both the TP-estimate based on the $\delta^{15}N$ values of Glu and Phe with canonical parameters for the metazoan food chain (i.e. $TP_{Glu/Phe}$; Chikaraishi et al. 2009) and the TP-estimate based on the $\delta^{15}N$ values of Ala and Phe with another set of parameters for the metazoan food chain potentially including a protozoan pathway (i.e. $TP_{Ala/Phe}$; Décima et al. 2017) were calculated for the targeted samples using the following equations, respectively:

$$TP_{Glu/Phe} = (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4) / 7.6 + 1 \quad (1)$$

$$TP_{Ala/Phe} = (\delta^{15}N_{Ala} - \delta^{15}N_{Phe} - 3.2) / 5.5 + 1 \quad (2)$$

RESULTS

Size-frequency distributions for ghost shrimp embryos and newly hatched zoeae I

The newly fertilized embryo-volume-frequency distribution and newly hatched zoea I-total length-frequency distribution of *Nihonotrypaea harmandi* in 2014 are shown in Fig. 2. Each frequency distribution comprised 2 cohorts (cohorts 1 and 2), to which normal distributions were fitted. The mean diameters of embryos (per female) ranged from 0.44 (short axis) to 0.52 mm (long axis). The estimated embryo volume ranged from 0.37 to 0.59 mm^3. Cohort 1 comprised 77% of all embryos, with mean (±SD) of 0.42 ± 0.02 mm^3. For cohort 2, the mean embryo volume was 0.50 ± 0.04 mm^3, which was 1.19 times greater than for cohort 1. The total length of zoea I ranged from 2.56 to 2.95 mm. Cohort 1 comprised 54% of all zoeae I, with mean of 2.70 ± 0.06 mm. For cohort 2, the mean total length was 2.87 ± 0.06 mm. The (mean total length)^3 was 1.20 times greater than that in cohort 1, where the cube is a measure for larval volume.

Water temperature and salinity in laboratory rearing tanks for ghost shrimp larvae

The time series of seawater temperature and salinity in the 2 rearing tanks for *N. harmandi* larvae through the whole period (05:00 h on 8 July to 09:00 h on 17 August in 2013) are shown in Fig. S1 in the Supplement. In rearing tank 1, the water temperature ranged from 20.9 to 23.1°C, with mean (±SD) of 22.2 ± 0.25°C. Salinity ranged from 32.5 to 34.1, with mean of 33.5 ± 0.2. In rearing tank 2, the water temperature ranged from 21.3 to 23.0°C, with mean of 22.3 ± 0.1°C. Salinity ranged from 32.3 to 33.8, with mean of 33.4 ± 0.2.

Development and survival of ghost shrimp larvae in laboratory rearing

At the time of daily exchange of water in the rearing tanks, the ‘old’ water was brownish in color, suggesting an excess ration of *Chaetoceros gracilis*. The daily occurrence of

393 decapodids of *N. harmandi* in rearing tanks 1 and 2 is shown in Fig. 3. During the rearing
394 period in each tank, there were 2 major cohorts (cohorts 1 and 2), to which normal distribution
395 curves were fitted. Decapodids emerged first on Day 26 in both tanks. In rearing tank 1, a total
396 of 175 decapodids occurred until Day 40, giving a survival rate of 3.9% of the initial number of
397 zoeae I from which the number of advanced-stage zoeae retrieved on the way to the decapodid
398 stage was subtracted. Cohort 1 comprised 30% of all decapodids, with mean occurrence in day
399 number being 28.7 ± 1.0. For cohort 2, the mean (±SD) occurrence as day number was 34.2 ±
400 2.6. In rearing tank 2, a total of 127 decapodids occurred until Day 40, giving a survival rate of
401 3.4%. Cohort 1 comprised 35% of all decapodids, with mean occurrence in day number being
402 28.8 ± 1.4. For cohort 2, the mean occurrence as day number was 35.3 ± 2.3.

403 ## TEF associated with diatom-feeding by ghost shrimp larvae

404 The SI values of the diet (diatom: *C. gracilis*) in the *N. harmandi* larval rearing
405 experiment were almost constant through the study period (mean ± SD, n = 17 values of $\delta^{13}C$ =
406 −22.7 ± 0.3‰ and $\delta^{15}N$ = −1.9 ± 0.3‰; Fig. 4), and these mean values were used for the
407 calculation of TEFs. The SI values in the zoeal whole-body tissues gradually shifted, with
408 development, from those in zoea I ($\delta^{13}C$ = −18.1 ± 0.1‰ and $\delta^{15}N$ = 7.1 ± 0.02‰, n = 3) to the
409 asymptotic values closer to that for diatoms. The SI values in the body tissue were −20.7‰ for
410 $\delta^{13}C$ and 1.0‰ for $\delta^{15}N$ (n = 1) at the zoea IV on Day 15 and reached a steady state at the
411 decapodid during Day 28 to Day 33 ($\delta^{13}C$ = −20.7 ± 0.3‰ and $\delta^{15}N$ = 0.0 ± 0.3‰, n = 15). The
412 SI values for the larvae that still remained at the zoeal stages (V and VI) in the tanks on Day 40
413 ($\delta^{13}C$ = −20.5 ± 0.3‰ and $\delta^{15}N$ = 0.0 ± 0.3‰, n = 3) were similar to those steady values. Thus,
414 the TEFs associated with the feeding of diatoms by *N. harmandi* zoeae was calculated as 2.0‰
415 for $\delta^{13}C$ and 1.9‰ for $\delta^{15}N$.

416 ## Measurement for shift in phytoplankton SI values with degradation

During the degradation experiment in June 2013, the mean SI values for the POM (fraction between 20 and 200 μm) from the chl maximum layers gradually decreased from Day 0 (mean [±SD] $\delta^{13}C$ = –20.0 ± 0.4‰, n = 3 and $\delta^{15}N$ = 5.6 to 5.7‰, n = 2) to Day 7 ($\delta^{13}C$ = –20.9 to –20.3‰, n = 2 and $\delta^{15}N$ = 4.9‰, n = 1) (Fig. 5). A similar decreasing tendency was observed in July 2013. The SI values for the POM decreased from Day 0 ($\delta^{13}C$ = –19.4 to –19.1‰, n = 2 and $\delta^{15}N$ = 6.2 to 6.4‰, n = 2) to Day 5 ($\delta^{13}C$ = –19.6 to –19.5‰, n = 2 and $\delta^{15}N$ = 5.2 to 5.6‰, n = 2). The average daily decreasing rates (‰ d^{-1}) were –0.09 for $\delta^{13}C$ and –0.11 for $\delta^{15}N$ during the 7 d in June, and –0.07 for $\delta^{13}C$ and –0.18 for $\delta^{15}N$ during the 5 d in July.

Estimation of TP for ghost shrimp larvae based on AA $\delta^{15}N$

The AA-specific $\delta^{15}N$ values of 2 sets of the *N. harmandi* zoea-VI samples were 2.3 and 2.9‰ for Phe, 14.7 and 15.2‰ for Ala, and 14.4 and 14.5‰ for Glu, respectively. The calculated mean TPs for ghost shrimp larvae were 2.1 for $TP_{Glu/Phe}$ and 2.7 for $TP_{Ala/Phe}$.

Taxonomic composition and standing crop of small-sized plankton in Amakusa-nada

Total abundance and total biovolume of nano- to micro-sized plankton in the 4 depth layers from the surface to 60 m at Stn A on 7 August 2012 ranged from 7.0×10^4 to 1.2×10^5 ind. l^{-1} and from 1.5×10^8 to 2.4×10^8 μm^3 l^{-1}, respectively, with values tending to decrease with depth (Fig. 6). Diatoms were dominant through the water column, both in abundance (47.3 to 55.3%) and biovolume (37.7 to 80.7%). Specifically, centric diatoms composed of Coscinodiscineae, Rhizosoleniineae, and Biddulphiineae accounted for 70.7 to 90.0% in abundance and 93.6 to 97.2% in biovolume of the diatoms. Dinoflagellates (11.0 to 27.7% in abundance and 9.5 to 23.1% in biovolume, mainly composed of Gymnodiniales) or ciliates (5.8

to 12.2% in abundance and 3.8 to 33.2% in biovolume, mainly composed of aloricate type) were subordinate.

Bulk SI values for ghost shrimp larvae and their potential food sources

The salinity averaged over the surface to 40 m depth at Stn A (Fig. 1) from CTD data in each cruise was lower in 2012 (mean [±SD]: 33.0 ± 0.5 in July and 32.6 ± 0.3 in August) than in 2013 (33.7 ± 0.2 in June, 33.6 ± 0.2 in July, and 33.4 ± 0.1 in August). Irrespective of the years, around the chl maximum layers at Stn A, higher chl *a* concentrations (>10 μg l^{-1}) were observed at 10 to 20 m depths in June and July, while lower peaks of 2 to 8 μg l^{-1} were observed at 30 to 50 m depths in August.

The SI values of biological samples and POM from Amakusa-nada were compiled separately for each of 2012 and 2013 (Fig. 7). The $\delta^{13}C$ in *N. harmandi* zoeal whole-body tissues preserved in formalin ($\delta^{13}C_{formalin}$) had lighter values than those in frozen (= 'intact') samples ($\delta^{13}C_{frozen}$) (i.e. mean [±SD] $\Delta\delta^{13}C$ [$\equiv \delta^{13}C_{formalin} - \delta^{13}C_{frozen}$] = −1.0 ± 0.2‰, n = 9, while $\delta^{15}N_{formalin}$ had heavier values than those in $\delta^{15}N_{frozen}$ i.e. $\Delta\delta^{15}N$ [$\equiv \delta^{15}N_{formalin} - \delta^{15}N_{frozen}$] = 0.5 ± 0.3‰, n = 9). Therefore, the $\delta^{13}C$ and $\delta^{15}N$ values for the zoeal whole-body tissues given in the food-chain diagram (Fig. 7) are those after the correction for the effect of formalin preservation by subtracting the above mean Δ-values from $\delta^{13}C_{formalin}$ or $\delta^{15}N_{formalin}$. The $\delta^{13}C$ and $\delta^{15}N$ values for other zooplankton whole-body tissues also designate those compensated ones.

In 2012, the mean $\delta^{13}C$ and $\delta^{15}N$ values in *N. harmandi* zoeal whole-body tissues at stages I and II inclusive were −18.3 ± 0.2‰ and 6.0 ± 0.2‰ (n = 7), respectively, and those values at stages III and IV inclusive had similar values ($\delta^{13}C$: −18.5 ± 0.3‰; $\delta^{15}N$: 6.3 ± 0.2‰, n = 6) (Fig. 7a). Most of the $\delta^{13}C$ and $\delta^{15}N$ values in the zoeae at stages V and VI inclusive showed lighter values (i.e. $\delta^{13}C$: −20.2 ± 0.3‰; $\delta^{15}N$: 5.7 ± 0.2‰, n = 5), which were

464 sufficiently distant from those at the earlier stages, though several latest-stage specimens (2 ind.
465 of zoea V and 1 ind. of zoea VI) took values closer to the earlier-stage values. The $\delta^{13}C$ and
466 $\delta^{15}N$ values for POM around the chl maximum layer showed large variations ranging from
467 −22.8 to −18.7‰ (−20.5 ± 1.3‰, n = 10), and from 4.8 to 6.2‰ (5.6 ± 0.4‰, n = 10),
468 respectively. The $\delta^{13}C$ and $\delta^{15}N$ values in the smaller zooplankton (0.3 to 1.2 mm in size) were
469 −19.9 ± 0.3‰ (n = 3) and 5.5 ± 0.2‰ (n = 3), respectively, while the larger zooplankton (1.2 to
470 3.5 mm in size) had relatively heavier values (−19.5 ± 0.2‰, n = 8 for $\delta^{13}C$ and 6.3 ± 0.3‰, n
471 = 8 for $\delta^{15}N$).

472 In 2013, the mean $\delta^{13}C$ and $\delta^{15}N$ values in *N. harmandi* zoeal whole-body tissues at
473 stages I and II inclusive were −17.6 and 6.1‰ (n = 1 for June and July each), respectively, and
474 those values gradually shifted to lighter ones with larval development; at stages III and IV
475 inclusive ($\delta^{13}C$: −18.3 ± 0.3‰; $\delta^{15}N$: 6.0 ± 0.2‰, n = 6) and at stages V and VI inclusive ($\delta^{13}C$:
476 −18.7 ± 0.2‰; $\delta^{15}N$: 5.7 ± 0.3‰, n = 10) (Fig. 7b). As in 2012, the $\delta^{13}C$ and $\delta^{15}N$ values for
477 POM in the chl maximum layer in 2013 showed large variations ranging from −22.1 to −19.4‰
478 (−20.5 ± 1.0‰, n = 5), and from 4.1 to 5.6‰ (5.0 ± 0.6‰, n = 5), respectively. The values for
479 POM in 2012 and 2013 inclusive were near to the previously reported values at 5 m depth on a
480 location 7.5 km east of Stn A in May and June 2004 ($\delta^{13}C$ = −21.3 ± 0.2‰ and $\delta^{15}N$ = 5.9 ±
481 0.3‰: Shimoda et al. 2007).

482 DISCUSSION

483 The present study was initiated primarily to fill a gap between the universally standard
484 laboratory diet (i.e. excess ration of a specific set of microzooplankton) for rearing decapod
485 crustacean larvae and the absence, or low availability, of nutritionally equivalent
486 microzooplankton in the coastal ocean water column containing plentiful phytoplankton and
487 their detritus (phytodetritus).

The larvae of *Nihonotrypaea harmandi* mass-reared at a water temperature of 22°C, with a diet consisting solely of pure-cultured nano-sized diatoms *Chaetoceros gracilis* in an excess ration, reached the first peak in decapodid occurrence on Day 29 and the second one on Days 34 and 35, and survived at rates of 3.4 to 3.9% from the initially housed zoeae I (Fig. 3). Zoeal bulk SI equilibration with regard to diatom SIs (Fig. 4) excludes the possibility of ingesting deposited animal material. The above-mentioned values are comparable with those obtained from the 2 previous rearing experiments under similar conditions to the present study (Tamaki et al. 2013). There, about 6600 newly hatched zoeae were housed in a 30 l tank and reared with a combination of *C. gracilis* (targeted only for zoea I), *Brachionus rotundiformis* (rotifer; provided for all zoeal stages), and *Artemia* sp. nauplii (targeted for zoeae III-VI), with each food item in an excess ration at 2 constant water temperatures. At 21°C, the first and second peaks in decapodid occurred on Day 30 (Tamaki et al. 2013) and Day 35 (A. Tamaki unpubl. data), respectively, with a combined retrieval rate of 7.35% (Tamaki et al. 2013). At 24°C, those peaks occurred on Day 30 (Tamaki et al. 2013) and Day 34 (A. Tamaki unpubl. data), with a combined retrieval rate of 4.7% (Tamaki et al. 2013). The 2 cohorts in the *N. harmandi* decapodids are likely to have originated from those cohorts in newly fertilized embryos and newly hatched zoeae I, for which a common ratio in the larger to smaller mean volumes existed (i.e. 1.2; Fig. 2). The larger eggs in *N. harmandi* occur around the time when females begin to participate in reproduction, and repeated egg laying with time brings about smaller eggs (A. Tamaki unpubl. data; cf. Kubo et al. 2006). One congeneric species, *N. japonica*, has a mean egg volume 1.5 to 2.0 times greater and a mean zoea I total length 1.11 times greater than those in *N. harmandi* (see Kubo et al. 2006 and Tamaki & Miyabe 2000, respectively); the difference in cubic total length is 1.37 times. The larval development of *N. japonica*, from zoea I through zoea II to zoea V to the first occurrence of decapodids, took 16 d in a laboratory rearing under similar conditions as in Tamaki et al. (2013) (Miyabe et al. 1998). Among decapod crustacean species, those with larger eggs tend to have greater yolk contents

514 (Wear 1974), which would lead to shorter larval developmental durations, including
515 callianassid shrimp species (Kubo et al. 2006). This might also be true for the intraspecific
516 difference in egg volume and larval developmental duration.

517 Of the 3 available studies about the successful rearing of larvae of the decapod
518 crustacean suborder Pleocyemata solely with phytoplankton, 2 were qualitative, in which
519 nano- to micro-sized diatoms and dinoflagellates (Atkins 1955) or green alga and diatom
520 (Bousquette 1980) were provided to pinnotherid brachyurans. The other one quantitatively
521 tested the effect of 3 diatom species with different sizes (250, 40, and 10 μm in φ) on the
522 brachyuran *Hyas coarctatus*, with only the largest species (*Biddulphia sinensis*: micro-size)
523 found to be a valid food but much less efficient than *Artemia* sp. nauplii (Harms & Seeger
524 1989). In that case, chain-forming in diatoms was regarded as essential for ingestion by the
525 zoeae. In the digestive tract contents of *N. harmandi* zoeae from Amakusa-nada, micro-sized
526 diatoms belonging to Rhizosoleniineae, Coscinodiscineae, Biddulphiineae, and Pennales were
527 found (Somiya et al. 2014). Each diatom frustule or piece was shorter than 25 μm in width and
528 height, while the length was not limited; smaller or slender diatoms might be ingested whole,
529 while larger or wider diatoms would be masticated by the mandible before ingestion. Cells of *C.*
530 *gracilis* used for the present study were nano-sized and not chain-forming, and *N. harmandi*
531 zoeae ingest them not individually but as a bolus accumulated by filter-feeding around the
532 mouthparts (R. Somiya pers. comm.). Such ingestion of lumps was observed for conspecific
533 decapodids and juveniles (Yokoyama et al. 2005, Tamaki et al. 2013). Under field conditions,
534 intraspecifically, zoeae of Pleocyemata species ingest a wide range of non-motile (and motile)
535 phytoplankton (and phytodetritus), from pico, through nano, to micro in size (Stickney &
536 Perkins 1981, Paul et al. 1989, Meyer-Harms & Harms 1993, Fileman et al. 2014). Such
537 indiscriminate ingestion of prey by those zoeae, including microzooplankton prey with limited
538 mobility used for the laboratory rearing, can be understood by widely known filter-feeding

habits of Pleocyemata larvae for any encountered prey of varying sizes (Rumrill et al. 1985, Factor & Dexter 1993, Crain 1999, Kiørboe 2011, Wirtz 2012).

The indiscriminate filter-feeding of decapod crustacean zoeae could support their growth and survival under non-motile prey-rich conditions in the dark (Harvey & Epifanio 1997, Hinz et al. 2001). In the water column of Amakusa-nada in the summer of 2006, most *N. harmandi* zoeae stayed below 20 m depth, where photon flux density was less than 2% of that at the surface, with the chl maximum layer at 22 to 24 m (Tamaki et al. 2010). In that water column in August 2012, diatoms were the predominant taxonomic group, accounting for 57 to 81% of the total biovolume of the potential prey assemblage between 20 and 40 m water depths (Fig. 6b). This tendency would be common in temperate coastal waters with higher nutrient concentrations during the summertime (Harvey et al. 1997, Furuya et al. 2003). Therefore, if *N. harmandi* zoeae indiscriminately ingest non-motile plankton by filter feeding in Amakusa-nada, diatoms and their sinking detritus would be the primary diet.

Although SI analysis can be used to provide strong evidence for the trophic status of meroplanktonic larvae of decapod crustaceans in the field, actual studies are scarce and limited to bulk analyses (Schwamborn et al. 1999, 2002). Of the present 2 TP estimates for *N. harmandi* zoeae from Amakusa-nada based on the $\delta^{15}N$ of amino acids (TP_{AAs}), one was 2.1 when using the most common measure suitable for metazoan food-chain ($TP_{Glu/Phe}$), while the other was 2.7 when using a measure incorporating a possible protistan grazing pathway ($TP_{Ala/Phe}$). Combination of these 2 estimates suggest that contribution of metazoans to the diet of those zoeae is minimum ($TP_{Glu/Phe} \approx 2$) and that the primary diet is a mixture of phytoplankton and heterotrophs mainly including protozoa ($TP_{Ala/Phe}$ = 2 to 3). The potentially mixed prey consisting of phytoplankton and protists was also reported for some suspension-feeding smaller holozooplankton based on the 2 TP_{AAs} (e.g. $TP_{Glu/Phe} \approx 2$ and $TP_{Ala/Phe}$ = 2.5 to 3.0 for copepodite-stage individuals of *Calanus pacificus* and juveniles of

564 *Euphausia pacifica* in Californian coastal waters; Décima et al. 2017); the body size of *N.*
565 *harmandi* zoeae (3 to 7 mm) is larger than that of these zooplankton. Protists constituted a part
566 of the diet for reared brachyuran larvae (Lehto et al. 1998, Hinz et al. 2001), and nano- and
567 pico-eukaryotes and ciliates were contained in the digestive tract contents of wild-caught
568 brachyuran and gebiidean larvae (Fileman et al. 2014). The setal structure and motion of
569 mouthpart appendages of *N. harmandi* zoeae are inefficient in collecting sparse tiny cells and
570 in grasping motile plankton such as free-living ciliates and dinoflagellates (Somiya et al. 2014,
571 R. Somiya unpubl. data). Generally, phytodetritus is regarded as a complex aggregate with
572 heterotrophs (Turner 2015), which can be large enough for decapod crustacean larvae to collect
573 as well as large diatoms. Thus, phytoplankton, diatoms in particular (Fig. 6), and their detritus
574 with associated heterotrophs including protozoa are estimated to be the most likely diet of *N.*
575 *harmandi* zoeae.

576 The bulk SI analysis provided another clue to support the estimated contribution of
577 phytodetritus to the *N. harmandi* larval diet. The bulk SI ($\delta^{13}C$ and δ^{15}) values for the
578 latest-stage zoeae (V and VI) in Amakusa-nada are expected to have been equilibrated with
579 those for their food sources, following the observation of the SIs in whole-body tissue of zoeae
580 fed cultured diatoms and their stabilization around Day 20 (Fig. 4). In fact, in the dual bulk SI
581 diagram comprising POM, zooplankton (only in 2012), and the zoeae, the SI values for the
582 zoeae I and II shifted to the lighter ones as they grew to the zoeae V and VI both in 2012 and
583 2013 (Fig. 7). The mean (±SD) $\delta^{15}N$ values for zoeae V and VI in 2012 (5.7 ± 0.2‰, excluding
584 outliers) were similar to those of the smaller zooplankton (5.5 ± 0.2‰), and even lighter than
585 those of the larger zooplankton (6.3 ± 0.3‰) (Fig. 7a). This suggests that copepod-dominated
586 zooplankton (0.3 to 3.5 mm in size) were not the potential food source for the *N. harmandi*
587 zoeae. A similar configuration of microzooplankton (presumed primary consumers) and
588 decapod crustacean zoeae in the dual bulk SI diagram was found for a plankton assemblage in

589 the coastal ocean off a mangrove estuary in northeastern Brazil (Schwamborn et al. 1999,
590 2002). Unexpectedly in the present study, the mean $\delta^{15}N$ value for the POM (5.7 ± 0.5‰) was
591 not far below but rather close to the $\delta^{15}N$ value for the zoeae V and VI. This similarity poses a
592 question on the status of POM (presumed phytoplankton) as the main food source for the zoeae,
593 considering isotopic discrimination across trophic steps. Firstly, POM is not composed of
594 phytoplankton only, but of a mixture of phytoplankton, phytodetritus, zooplankton debris, and
595 fecal pellets (Lee 2002, Volkman & Tanoue 2002). With POM being such a complex mixture,
596 the $\delta^{15}N$ value might be lighter for live phytoplankton per se than for POM. Secondly, even if
597 the main constituent of POM from the chl maximum layers is live phytoplankton, its degraded
598 form (i.e. phytodetritus including heterotrophs), with altered SI values, could be the principal
599 diet of the *N. harmandi* zoeae. These larvae might selectively feed on sinking aggregates
600 composed of degraded lumps of diatoms with heterotrophs, which could be more easily
601 collected and ingested than loose cells.

602 The analyses of the dual bulk SI diagrams indicated that phytodetritus was likely an
603 important food source for *N. harmandi* zoeae, based on the SI shifts in POM during
604 degradation and larval TEFs (Fig. 7). Those zoeae stay mainly at 30 to 50 m depths in
605 Amakusa-nada (Tamaki et al. 2010), where sinking phytodetritus originated from the upper chl
606 maximum layer (usually 10 to 20 m depths except for August, with 30 to 40 m depths) may be
607 abundant. A potential daily shift in the SI values of POM derived from that layer, with
608 microbial degradation, is estimated at –0.07 to –0.09‰ d^{-1} for $\delta^{13}C$ and –0.11 to –0.18‰ d^{-1}
609 for $\delta^{15}N$, based on the 2 incubation experiments (Fig. 5). These alteration rates are comparable
610 to those reported in Lehmann et al. (2002), in which diatom-dominated POM was incubated
611 under different redox conditions (–0.06 to –0.13‰ d^{-1} for $\delta^{13}C$ and –0.10 to –0.12‰ d^{-1} for
612 $\delta^{15}N$; calculated here from those for the initial 10 d of the original 111 d incubation). Assuming
613 that live phytoplankton at the chl maximum layer in Amakusa-nada sink for a distance of 25 m

614 in 7 d at an average speed of 3.5 m d^{-1} (cf. Kriest & Oschlies 2008), with the corresponding
615 SI-value alterations ($\Delta\delta^{13}C$ = –0.5 to –0.6‰ wk^{-1}; $\Delta\delta^{15}N$ = –0.8 to –1.3‰ wk^{-1}), the range of
616 the SI values for the expected degraded POM will be located at the lighter position than that for
617 the measured POM with large variations. In 2012, the expected $\delta^{13}C$ and $\delta^{15}N$ values of the
618 food source for *N. harmandi* zoeae V and VI inclusive based on their whole-body (including
619 exoskeleton)-specific TEFs (2.0‰ for $\delta^{13}C$; 1.9‰ for $\delta^{15}N$; Fig. 4) lay close to the edge lines
620 of the larger SI-box for the expected degraded-POM (expected FS [food source]-1 in Fig. 7a).
621 In a previous companion rearing experiment for *N. harmandi* juveniles, Yokoyama et al.
622 (2005) reported that TEFs for the whole body (–1.7‰ for $\delta^{13}C$; 2.3‰ for $\delta^{15}N$) were lower
623 than those for the muscle, probably due to the effect of chitin in the exoskeleton; no lipid
624 removal was made as in the present study. Based on these specific TEFs, there was no
625 candidate organic matter for the zoeal food sources (expected FS-2). Thus, it appears
626 indispensable to obtain the TEFs that are specific to the zoeal whole body, with its exoskeleton
627 probably not so fully developed as in juvenile exoskeleton.

628 The configuration of POM, presumed degraded-POM, and *N. harmandi* zoeae in the
629 dual bulk SI diagram for 2013 was largely similar to that for 2012, though the $\delta^{13}C$ values for
630 zoeae V and VI inclusive in 2012 were lighter by about 1.5‰ (Fig. 7b). One reason for the
631 latter might be a higher contribution of terrestrial dissolved inorganic carbon to primary
632 production, with relatively lighter $\delta^{13}C$ values than those in seawater (e.g. Boutton 1991). The
633 cumulative rainfall recorded at a meteorological observatory in Kumamoto Prefecture (32.813°
634 N, 130.707° E), located near Ariake Sound, during the rainy season of Japan (June and July)
635 was 3 times higher in 2012 than in 2013 (1200 vs. 430 mm; Japan Meteorological Agency Past
636 Weather Data; www.data.jma.go.jp/obd/stats/etrn/index.php). The average discharge of the
637 nearby largest river (Chikugo River emptying into the innermost Ariake Sound, with a 35%
638 watershed area in the sound; Iyama 2007) in this season were also 3 times higher in 2012 (493

639 vs. 163 m^3 s^{-1} at Senoshita station; Ministry of Land, Infrastructure, Transport and Tourism
640 Water Information System; http://www1.river.go.jp/). In accordance with this, the salinity in
641 the water column at Stn A in Amakusa-nada was lower in 2012 (see 'Results'). Although such
642 river discharge may not have been clearly reflected in the $\delta^{13}C$ of POM in our limited survey
643 results (i.e. 2 to 3 time water samplings for 1 d each month), its signature could have been
644 manifested over time in the $\delta^{13}C$ of *N. harmandi* zoeae via primary production. Despite these
645 yearly meteorological differences, the plot for the mean SIs of the expected food source for the
646 latest-stage zoeae of *N. harmandi* (FS-1) lying close to the edge (in 2012) or inside (in 2013) of
647 the larger SI box for the presumed degraded-POM provides robust evidence for phytoplankton
648 (especially diatom)-derived detritus including heterotrophs as the principal potential diet.

649 The bulk $\delta^{13}C$ and $\delta^{15}N$ analysis for *N. harmandi* adults from the Tomioka sandflat
650 revealed that their diet was phytoplankton (as POM) from the coastal ocean and benthic
651 microalgae on the sandflat (Shimoda et al. 2007). Since the constant deposition of live
652 microalgae into the shrimp burrow makes their density high, the SI signature of food sources in
653 shrimp bodies appears to be unambiguous. In contrast, the density of live phytoplankton is
654 lower in the water column, especially for herbivorous zooplankton staying below the chl
655 maximum layer. At 10s of meter water depths in the inner-shelf coastal ocean, phytodetritus
656 (enriched with heterotrophs) would serve as the seasonally most abundant food source for
657 filter-feeding larvae of decapod crustaceans, probably being more dense than live
658 phytoplankton (time-integrated vs. instantaneous resources).

659 ## CONCLUSIONS

660 The present study has demonstrated that the carnivorous feeding habit of decapod
661 crustacean larvae preying on microzooplankton such as brine shrimp nauplii under laboratory
662 culture is not necessarily applicable to their habit in the field, raising a possibility that
663 phytoplankton-based diet is more common in meroplanktonic larvae of the suborder

664 Pleocyemata than previously thought, especially in coastal oceans connected to estuaries with
665 high primary production. In this demonstration, a combined approach (rearing with
666 pure-cultured phytoplankton, field surveys of nano- to micro-sized plankton assemblage, TP
667 analysis based on AA $\delta^{15}N$, determination of species-specific TEFs, and bulk SI analysis)
668 proved useful to estimate diatom-dominated phytoplankton and their detritus with heterotrophs
669 including protozoa as the principal diet of meroplanktonic larvae. The possible trophic status of
670 those planktotrophic larvae as the consumer relying mainly on phytoplankton would be
671 relevant to such aspects as (1) bottom-up effects of primary producers on the survival of those
672 larvae (Thorson 1950, Stickney & Perkins 1981, Olson & Olson 1989, Shirley & Shirley 1989,
673 Kirby et al. 2008), (2) reciprocal impact from those larvae to primary producers (Fileman et al.
674 2014), (3) vertical migration of those larvae in relation to phytoplankton and phytodetritus
675 distribution in the water column and its consequent horizontal transport (Pearre 2003,
676 Woodson & McManus 2007, Tamaki et al. 2010), and (4) biogeography of plankto- and
677 lecitho-trophic larval-type distribution with chlorophyll concentration distribution in the global
678 sea surface (Thorson 1950, Marshall et al. 2012).

679 *Acknowledgements.* We thank the captain and crews of the T/V 'Kakuyo-Maru', Nagasaki
680 University for support in sampling. H. Matsuo, H. Uchida, and S. Ohashi assisted with larval
681 culture, and R. Somiya, K. Okamura, K. Kiyama, D. Hiramatsu, and H. Goto assisted with
682 either sample collection, processing, or analysis. S. Takeda provided chl *a* concentration data
683 from the shared cruises. The water-depth data were provided by the Hydrographic and
684 Oceanographic Department, Japan Coast Guard. This research was supported by the
685 Environment Research and Technology Development Fund (4D-1104) of the Ministry of the
686 Environment, Japan and the Japan Society for the Promotion of Science Grant-in-Aid for
687 Scientific Research JP26440244 to A.T. Stable isotope analyses were partly supported by JST
688 CREST Grant Number JPMJCR13A3, Japan.

LITERATURE CITED

<jrn>Aizawa Y, Takiguchi N (1999) Consideration of the methods for estimating the age-composition from the length frequency data with MS-Excel. Bull Jpn Soc Fish Oceanogr 63:205–214 (in Japanese)</jrn>

<edb>Anger K (2001) The biology of decapod crustacean larvae. In: Vonk R (ed) Crustacean issues, Vol 14. A.A. Balkema, Rotterdam</edb>

Atkins D (1955) The post-embryonic development of British *Pinnotheres* (Crustacea). Proc. Zool. Soc. Lond. 124:687-715

Bousquette GD (1980) The larval development of *Pinnixa longipes* (Lockington, 1877) (Brachyura, Pinnotheridae), reared in the laboratory. Biol. Bull. 159:592-605

<edb>Boutton TW (1991) Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. In: Coleman DC, Fry B (eds) Carbon isotope techniques. Academic Press, San Diego, CA, p 173–185</edb>

<jrn>Burnett N, Sulkin S (2007) Characteristics of feeding on dinoflagellates by newly hatched larval crabs. Mar Biol 151:851–861 doi:10.1007/s00227-006-0531-x</jrn>

<jrn>Calado R, Pimentel T, Pochelon P, Olaguer-Feliú AO, Queiroga H (2010) Effect of food deprivation in late larval development and early benthic life of temperate marine coastal and estuarine caridean shrimp. J Exp Mar Biol Ecol 384:107–112 doi:10.1016/j.jembe.2010.01.003</jrn>

<jrn>Chikaraishi Y, Ogawa NO, Kashiyama Y, Takano Y and others (2009) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol Oceanogr Methods 7:740–750 doi:10.4319/lom.2009.7.740</jrn>

711 <jrn>Crain JA (1999) Functional morphology of prey ingestion by *Placetron wosnessenskii*
712 Schalfeew zoeae (Crustacea: Anomura: Lithodidae). Biol Bull (Woods Hole) 197:207–218
713 PubMed doi:10.2307/1542616</jrn>

714 <edb>Day R, McEdward L (1984) Aspects of the physiology and ecology of pelagic larvae of
715 marine benthic invertebrates. In: Steidinger, KA, Walker LM (eds) Marine plankton life cycle
716 strategies. CRC Press, Boca Raton, FL, p 93–120</edb>

717 <jrn>Décima M, Landry MR, Bradley CJ, Fogel ML (2017) Alanine δ^{15}N trophic fractionation
718 in heterotrophic protists. Limnol Oceanogr 62:2308–2322 doi:10.1002/lno.10567</jrn>

719 <jrn>DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in
720 animals. Geochim Cosmochim Acta 42:495–506 doi:10.1016/0016-7037(78)90199-0</jrn>

721 <edb>Dworschak PC, Felder DL, Tudge CC (2012) Infraorders Axiidea de Saint Laurent,
722 1979 and Gebiidea de Saint Laurent, 1979 (formerly known collectively as Thalassinidea). In:
723 Schram FR, von Vaupel Klein JC, Charmantier-Daures M, Forest J (eds) Treatise on zoology:
724 anatomy, taxonomy, biology. The Crustacea. Complementary to the volumes translated from
725 the French of the Traité de Zoologie, Vol 9, Part B. Eucarida: Decapoda: Astacidea p.p.
726 (Enoplometopoidea, Nephropoidea), Glypheidea, Axiidea, Gebiidea, and Anomura.
727 Koninklijke Brill, Leiden, p 109–219</edb>

728 <jrn>Emmerson WD (1985) Fecundity, larval rearing and laboratory growth of *Palaemon*
729 *pacificus* (Stimpson) (Decapoda, Palaemonidae). Crustaceana 49:277–289
730 doi:10.1163/156854085X00594</jrn>

731 <jrn>Factor JR, Dexter BL (1993) Suspension feeding in larval crabs (*Carcinus maenas*). J
732 Mar Biol Assoc UK 73:207–211 doi:10.1017/S0025315400032732</jrn>

733 <jrn>Fileman ES, Lindeque PK, Harmer RA, Halsband C, Atkinson A (2014) Feeding rates
734 and prey selectivity of planktonic decapod larvae in the Western English Channel. Mar Biol
735 161:2479–2494 doi:10.1007/s00227-014-2520-9</jrn>

736 <jrn>Furuya K, Hayashi M, Yabushita Y, Ishikawa A (2003) Phytoplankton dynamics in the
737 East China Sea in spring and summer as revealed by HPLC-derived pigment signatures. Deep
738 Sea Res II 50:367–387 doi:10.1016/S0967-0645(02)00460-5</jrn>

739 <edb>Guillard RRL (1978) Counting slides. In: Sournia A (ed) Monographs on oceanographic
740 methodology, Vol 6: phytoplankton manual. UNESCO, Paris, p 182−189</edb>

741 <jrn>Gutiérrez-Rodríguez A, Décima M, Popp BN, Landry MR (2014) Isotopic invisibility of
742 protozoan trophic steps in marine food webs. Limnol Oceanogr 59:1590–1598
743 doi:10.4319/lo.2014.59.5.1590</jrn>

744 <jrn>Harms J, Seeger B (1989) Larval development and survival in seven decapod species
745 (Crustacea) in relation to laboratory diet. J Exp Mar Biol Ecol 133:129–139
746 doi:10.1016/0022-0981(89)90162-7</jrn>

747 <jrn>Harms J, Anger K, Klaus S, Seeger B (1991) Nutritional effects on ingestion rate,
748 digestive enzyme activity, growth, and biochemical composition of *Hyas araneus* L.
749 (Decapoda: Majidae) larvae. J Exp Mar Biol Ecol 145:233–265
750 doi:10.1016/0022-0981(91)90178-Y</jrn>

751 <jrn>Harms J, Meyer-Harms B, Dawirs RR, Anger K (1994) Growth and physiology of
752 *Carcinus maenas* (Decapoda, Portunidae) larvae in the field and in laboratory experiments.
753 Mar Ecol Prog Ser 108:107–118 doi:10.3354/meps108107</jrn>

754 <conf>Hartman MC, Letterman GR (1978) An evaluation of three species of diatoms as food
755 for *Cancer magister* larvae. Proc Annu Meet World Maric Soc 9:271−276</conf>

756 <jrn>Harvey EA, Epifanio CE (1997) Prey selection by larvae of the common mud crab
757 *Panopeus herbstii* Milne-Edwards. J Exp Mar Biol Ecol 217:79–91
758 doi:10.1016/S0022-0981(97)00045-2</jrn>

759 <jrn>Harvey M, Therriault JC, Simard N (1997) Late-summer distribution of phytoplankton in
760 relation to water mass characteristics in Hudson Bay and Hudson Strait (Canada). Can J Fish
761 Aquat Sci 54:1937–1952 doi:10.1139/f97-099</jrn>

762 <jrn>Hasselblad V (1966) Estimation of parameters for a mixture of normal distributions.
763 Technometrics 8:431–444 doi:10.1080/00401706.1966.10490375</jrn>

764 <jrn>Hinz S, Sulkin S, Strom S, Testermann J (2001) Discrimination in ingestion of protistan
765 prey by larval crabs. Mar Ecol Prog Ser 222:155–162 doi:10.3354/meps222155</jrn>

766 <jrn>Incze LS, Paul AJ (1983) Grazing and predation as related to energy needs of stage I
767 zoeae of the tanner crab *Chionoecetes bairdi* (Brachyura, Majidae). Biol Bull (Woods Hole)
768 165:197–208 doi:10.2307/1541364</jrn>

769 <jrn>Ishikawa NF, Kato Y, Togashi H, Yoshimura M, Yoshimizu C, Okuda N, Tayasu I
770 (2014) Stable nitrogen isotopic composition of amino acids reveals food web structure in
771 stream ecosystems. Oecologia 175:911–922 PubMed doi:10.1007/s00442-014-2936-4</jrn>

772 <jrn>Iyama S (2007) Securing of water volume for environmental conservation of the Chikugo
773 River and the Ariake Sea. Bull Jpn Soc Sci Fish 73:108–111 (in Japanese)
774 doi:10.2331/suisan.73.108</jrn>

775 <jrn>Johnson GE, Gonor JJ (1982) The tidal exchange of *Callianassa californiensis*
776 (Crustacea, Decapoda) larvae between the ocean and the Salmon River estuary, Oregon. Estuar
777 Coast Shelf Sci 14:501–516 doi:10.1016/S0302-3524(82)80073-X</jrn>

778 <jrn>Kiørboe T (2011) How zooplankton feed: mechanisms, traits and trade-offs. Biol Rev
779 Camb Philos Soc 86:311–339 PubMed doi:10.1111/j.1469-185X.2010.00148.x</jrn>

780 <jrn>Kirby RR, Beaugrand G, Lindley JA (2008) Climate-induced effects on the
781 meroplankton and the benthic–pelagic ecology of the North Sea. Limnol Oceanogr
782 53:1805–1815 doi:10.4319/lo.2008.53.5.1805</jrn>

783 <jrn>Konishi K, Sakami T, Fujinami Y (1997) An attempt to estimate the amount of
784 microalgae ingestion by crab larvae. Cancer 6:3–7 (in Japanese)</jrn>

785 <conf>Konishi K, Fukuda Y, Quintana RR (1999) The larval development of the
786 mud-burrowing shrimp *Callianassa* sp. under laboratory conditions (Decapoda, Thalassinidea,
787 Callianassidae). In: Schram FR, von Vaupel Klein JC (eds) Crustaceans and the biodiversity
788 crisis. Proc 4th Int Crustacean Congr, Vol 1. Koninklijke Brill, Leiden, p 781–804</conf>

789 <jrn>Kornienko ES, Korn OM, Golubinskaya DD (2015) The number of zoeal stages in larval
790 development of *Nihonotrypaea petalura* (Stimpson, 1860) (Decapoda: Axiidea:
791 Callianassidae) from Russian waters of the Sea of Japan. Zootaxa 3919:343–361 PubMed
792 doi:10.11646/zootaxa.3919.2.7</jrn>

793 <jrn>Kriest I, Oschlies A (2008) On the treatment of particulate organic matter sinking in
794 large-scale models of marine biogeochemical cycles. Biogeosciences 5:55–72
795 doi:10.5194/bg-5-55-2008</jrn>

796 <jrn>Kubo K, Shimoda K, Tamaki A (2006) Egg size and clutch size in three species of
797 *Nihonotrypaea* (Decapoda: Thalassinidea: Callianassidae) from western Kyushu, Japan. J Mar
798 Biol Assoc UK 86:103–111 doi:10.1017/S0025315406012902</jrn>

799 <jrn>Landry MR, Décima M (2017) Protistan microzooplankton and the trophic position of
800 tuna: quantifying the trophic link between micro- and mesozooplankton in marine foodwebs.
801 ICES J Mar Sci 74:1885–1892</jrn>

802 <jrn>Lebour MV (1922) The food of plankton organisms. J Mar Biol Assoc UK 12:644–677
803 doi:10.1017/S0025315400009681</jrn>

804 <edb>Lee C (2002) Particulate organic matter composition and fluxes in the sea. In: Gianguzza
805 A, Pelizzetti E, Sammartano S (eds) Chemistry of marine water and sediments. Environmental
806 Science. Springer, Berlin, p 125–146</edb>
807 Lehmann MF, Bernasconi SM, Barbieri A, McKenzie JA (2002) Preservation of organic
808 matter and alteration of its carbon and nitrogen isotope composition during simulated and in
809 situ early sedimentary diagenesis. Geochim Cosmochim AC 66:3573-3584.
810 <jrn>Lehto J, Sulkin S, Strom S, Johnson D (1998) Protists and detrital particles as prey for the
811 first larval stage of the brachyuran crab, *Hemigrapsus oregonensis.* J Exp Mar Biol Ecol
812 230:213–224 doi:10.1016/S0022-0981(98)00074-4</jrn>
813 <jrn>Marshall DJ, Krug PJ, Kupriyanova EK, Byrne M, Emlet RB (2012) The biogeography
814 of marine invertebrate life histories. Annu Rev Ecol Evol Syst 43:97–114
815 doi:10.1146/annurev-ecolsys-102710-145004</jrn>
816 <jrn>Mascetti P, Wehrtmann IS (1996) Aspects of the reproductive biology of *Petrolisthes*
817 *laevigatus* (Guérin, 1835) (Decapoda, Anomura, Porcellanidae). Part III: Effects of starvation
818 and different types of diet on larval development under laboratory conditions. Arch Fish Mar
819 Res 43:159–170</jrn>
820 <jrn>McClelland JW, Montoya JP (2002) Trophic relationships and the nitrogen isotopic
821 composition of amino acids in plankton. Ecology 83:2173–2180
822 doi:10.1890/0012-9658(2002)083[2173:TRATNI]2.0.CO;2</jrn>
823 <edb>McConaugha JR (1985) Nutrition and larval growth. In: Wenner AM (ed) Crustacean
824 issues 2: larval growth. A.A. Balkema, Rotterdam, p 127–154</edb>
825 <jrn>Metaxas A, Saunders M (2009) Quantifying the 'bio-' components in biophysical models
826 of larval transport in marine benthic invertebrates: advances and pitfalls. Biol Bull (Woods
827 Hole) 216:257–272 PubMed doi:10.1086/BBLv216n3p257</jrn>

828 <jrn>Meyer-Harms B, Harms J (1993) Detection of phytoplankton pigments by HPLC in *Hyas*
829 *araneus* larvae (Crustacea, Decapoda): comparison of field and laboratory samples. Neth J Sea
830 Res 31:153–161 doi:10.1016/0077-7579(93)90005-D</jrn>

831 <jrn>Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further
832 evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta
833 48:1135–1140 doi:10.1016/0016-7037(84)90204-7</jrn>

834 <jrn>Miyabe S, Konishi K, Fukuda Y, Tamaki A (1998) The complete larval development of
835 the ghost shrimp, *Callianassa japonica* Ortmann, 1891 (Decapoda: Thalassinidea:
836 Callianassidae), reared in the laboratory. Crustac Res 27:101–121
837 doi:10.18353/crustacea.27.0_101</jrn>

838 <edb>Ogawa NO, Nagata T, Kitazato H, Ohkouchi N (2010) Ultra-sensitive elemental
839 analyzer/isotope ratio mass spectrometer for stable nitrogen and carbon isotopic analyses. In:
840 Ohkouchi N, Tayasu I, Koba K (eds) Earth, life, and isotopes. Kyoto University Press, Kyoto, p
841 339–353</edb>

842 <jrn>Ohkouchi N, Chikaraishi Y, Close HG, Fry B and others (2017) Advances in the
843 application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies.
844 Org Geochem 113:150–174 doi:10.1016/j.orggeochem.2017.07.009</jrn>

845 <jrn>Olson RR, Olson MH (1989) Food limitation of planktotrophic marine invertebrate
846 larvae: Does it control recruitment success? Annu Rev Ecol Syst 20:225–247
847 doi:10.1146/annurev.es.20.110189.001301</jrn>

848 <jrn>Paul AJ, Paul JM, Coyle KO (1989) Energy sources for first-feeding zoeae of king crab
849 *Paralithodes camtschatica* (Tilesius) (Decapoda, Lithodidae). J Exp Mar Biol Ecol 130:55–69
850 doi:10.1016/0022-0981(89)90018-X</jrn>

851 <jrn>Pearre S Jr (2003) Eat and run? The hunger/satiation hypothesis in vertical migration:
852 history, evidence and consequences. Biol Rev Camb Philos Soc 78:1–79 PubMed
853 doi:10.1017/S146479310200595X</jrn>

854 <jrn>Perez MF, Sulkin SD (2005) Palatability of autotrophic dinoflagellates to newly hatched
855 larval crabs. Mar Biol 146:771–780 doi:10.1007/s00227-004-1482-8</jrn>

856 <jrn>Pillay D, Branch GM (2011) Bioengineering effects of burrowing thalassinidean shrimps
857 on marine soft-bottom ecosystems. Oceanogr Mar Biol Annu Rev 49:137–192</jrn>

858 <jrn>Pohle G, Santana W, Jansen G, Greenlaw M (2011) Plankton-caught zoeal stages and
859 megalopa of the lobster shrimp *Axius serratus* (Decapoda: Axiidae) from the Bay of Fundy,
860 Canada, with a summary of axiidean and gebiidean literature on larval descriptions. J Crustac
861 Biol 31:82–99 doi:10.1651/10-3321.1</jrn>

862 <jrn>Preston NP, Burford MA, Coman FE, Rothlisberg PC (1992) Natural diet of larval
863 *Penaeus merguiensis* (Decapoda: Penaeidae) and its effect on survival. Mar Biol
864 113:181–191</jrn>

865 <jrn>Rumrill SS, Pennington JT, Chia FS (1985) Differential susceptibility of marine
866 invertebrate larvae: laboratory predation of sand dollar, *Dendraster excentricus* (Eschscholtz),
867 embryos and larvae by zoeae of the red crab, *Cancer productus* Randall. J Exp Mar Biol Ecol
868 90:193–208 doi:10.1016/0022-0981(85)90166-2</jrn>

869 <jrn>Schwamborn R, Voss M, Ekau W, Saint-Paul U (1999) Stable isotope composition of
870 particulate organic matter and zooplankton in northeast Brazilian shelf waters. Arch Fish Mar
871 Res 47:201–210</jrn>

872 <jrn>Schwamborn R, Ekau W, Voss M, Saint-Paul U (2002) How important are mangroves as
873 a carbon source for decapod crustacean larvae in a tropical estuary? Mar Ecol Prog Ser
874 229:195–205 doi:10.3354/meps229195</jrn>

875 <jrn>Schwamborn R, Ekau W, Silva AP, Schwamborn SHL, Silva TA, Neumann-Leitão S,
876 Saint-Paul U (2006) Ingestion of large centric diatoms, mangrove detritus, and zooplankton by
877 zoeae of *Aratus pisonii* (Crustacea: Brachyura: Grapsidae). Hydrobiologia 560:1–13
878 doi:10.1007/s10750-005-0988-5</jrn>

879 <jrn>Shaber K, Sulkin S (2007) Feeding on dinoflagellates by intermediate and late stage crab
880 zoeae raised in the laboratory and collected from the field. J Exp Mar Biol Ecol 340:149–159
881 doi:10.1016/j.jembe.2006.09.011</jrn>

882 <jrn>Shimoda K, Aramaki Y, Nasuda J, Yokoyama H, Ishihi Y, Tamaki A (2007) Food
883 sources for three species of *Nihonotrypaea* (Decapoda: Thalassinidea: Callianassidae) from
884 western Kyushu, Japan, as determined by carbon and nitrogen stable isotope analysis. J Exp
885 Mar Biol Ecol 342:292–312 doi:10.1016/j.jembe.2006.11.003</jrn>

886 Shirley SM, Shirley TC (1989) Interannual variability in density, timing and survival of
887 Alaskan red king crab Paralithodes camtschatica larvae. Mar Ecol Prog Ser 54:51-59

888 <jrn>Somiya R, Suzuki T, Tamaki A (2014) Mouthpart morphology and wild diet of zoeae of
889 the ghost shrimp, *Nihonotrypaea harmandi* (Decapoda: Axiidea: Callianassidae). J Crustac
890 Biol 34:300–308 doi:10.1163/1937240X-00002237</jrn>

891 <jrn>Stickney AP, Perkins HC (1981) Observations on the food of the larvae of the northern
892 shrimp, *Pandalus borealis* Kröyer (Decapoda, Caridea). Crustaceana 40:36–49
893 doi:10.1163/156854081X00381</jrn>

894 <jrn>Sulkin S, Lehto J, Strom S, Hutchinson D (1998) Nutritional role of protists in the diet of
895 first stage larvae of the Dungeness crab *Cancer magister.* Mar Ecol Prog Ser 169:237–242
896 doi:10.3354/meps169237</jrn>

897 <jrn>Tamaki A, Harada K (2005) Alongshore configuration and size of local populations of
898 the callianassid shrimp *Nihonotrypaea harmandi* (Bouvier, 1901) (Decapoda: Thalassinidea)

899 in the Ariake-Sound estuarine system, Kyushu, Japan. Crustac Res 34:65–86
900 doi:10.18353/crustacea.34.0_65</jrn>

901 <unknown>Tamaki A, Miyabe S (2000) Larval abundance patterns for three species of
902 *Nihonotrypaea* (Decapoda: Thalassinidea: Callianassidae) along an estuary-to-open-sea
903 gradient in western Kyushu, Japan. J Crustacean Biol 20 (Spec Number
904 2):182–191</unknown>

905 <jrn>Tamaki A, Takeuchi S (2016) Persistence, extinction, and recolonization of an epibenthic
906 gastropod population on an intertidal sandflat: 35-y contingent history of a key species of the
907 benthic community in metapopulation and metacommunity contexts. J Shellfish Res
908 35:921–967 doi:10.2983/035.035.0419</jrn>

909 <jrn>Tamaki A, Itoh J, Kubo K (1999) Distributions of three species of *Nihonotrypaea*
910 (Decapoda: Thalassinidea: Callianassidae) in intertidal habitats along an estuary to open-sea
911 gradient in western Kyushu, Japan. Crustac Res 28:37–51
912 doi:10.18353/crustacea.28.0_37</jrn>

913 <jrn>Tamaki A, Mandal S, Agata Y, Aoki I and others (2010) Complex vertical migration of
914 larvae of the ghost shrimp, *Nihonotrypaea harmandi*, in inner shelf waters of western Kyushu,
915 Japan. Estuar Coast Shelf Sci 86:125–136 doi:10.1016/j.ecss.2009.11.005</jrn>

916 <jrn>Tamaki A, Saitoh Y, Itoh J, Hongo Y, Sen-ju S, Takeuchi S, Ohashi S (2013)
917 Morphological character changes through decapodid-stage larva and juveniles in the ghost
918 shrimp *Nihonotrypaea harmandi* from western Kyushu, Japan: clues for inferring pre- and
919 post-settlement states and processes. J Exp Mar Biol Ecol 443:90–113
920 doi:10.1016/j.jembe.2013.02.038</jrn>

921 <jrn>Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biol
922 Rev Camb Philos Soc 25:1–45 PubMed doi:10.1111/j.1469-185X.1950.tb00585.x</jrn>

923 <jrn>Turner JT (2015) Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's
924 biological pump. Prog Oceanogr 130:205–248 doi:10.1016/j.pocean.2014.08.005</jrn>

925 <jrn>Volkman JK, Tanoue E (2002) Chemical and biological studies of particulate organic
926 matter in the ocean. J Oceanogr 58:265–279 doi:10.1023/A:1015809708632</jrn>

927 <jrn>Wear RG (1974) Incubation in British decapod Crustacea, and the effects of temperature
928 on the rate and success of embryonic development. J Mar Biol Assoc UK 54:745–762
929 doi:10.1017/S0025315400022918</jrn>

930 <jrn>Wirtz KW (2012) Who is eating whom? Morphology and feeding type determine the size
931 relation between planktonic predators and their ideal prey. Mar Ecol Prog Ser 445:1–12
932 doi:10.3354/meps09502</jrn>

933 <jrn>Woodson CB, McManus MA (2007) Foraging behavior can influence dispersal of marine
934 organisms. Limnol Oceanogr 52:2701–2709 doi:10.4319/lo.2007.52.6.2701</jrn>

935 <jrn>Yannicelli B, Castro LR, Schneider W, Sobarzo M (2006) Crustacean larvae distribution
936 in the coastal upwelling zone off Central Chile. Mar Ecol Prog Ser 319:175–189
937 doi:10.3354/meps319175</jrn>

938 <edb>Yano I (2005) Penaeid shrimp. In: Mori K (ed) Fishery enhancement and aquaculture
939 systems, Vol. 3. Kouseisha-Kouseikaku, Tokyo, p 299–328 (in Japanese)</edb>

940 <jrn>Yokoyama H, Tamaki A, Harada K, Shimoda K, Koyama K, Ishihi Y (2005) Variability
941 of diet-tissue isotopic fractionation in estuarine macrobenthos. Mar Ecol Prog Ser
942 296:115–128 doi:10.3354/meps296115</jrn>

943

944

945 Table 1. Partially valid phytoplanktonic food items for the development of reared larvae in

946 groups of the suborder Pleocyemata

Food items	Groups in Pleocyemata	Reference(s)
Diatoms	Brachyurans and/or anomurans	Hartman & Letterman (1978), Harms & Seeger (1989), Paul et al. (1989), Harms et al. (1991, 1994), Konishi et al. (1997)
Diatoms	Carideans	Stickney & Perkins (1981), Emmerson (1985)
Diatoms and dinoflagellates	Brachyurans	Incze & Paul (1983), Meyer-Harms & Harms (1993)
Dinoflagellates	Brachyurans	Perez & Sulkin (2005), Burnett & Sulkin (2007), Shaber & Sulkin (2007)
Dinoflagellates	Brachyurans and/or anomurans	Hinz et al. (2001)
Dinoflagellates and green alga	Brachyurans	Sulkin et al. (1998)
Dinoflagellates, green alga, and seagrass detritus	Brachyurans	Lehto et al. (1998)
Haptophyte alga	Brachyurans and/or anomurans	Mascetti & Wehrtmann (1996)

947

948

949 Fig. 1. Study region and location of tidal flats along the shoreline in mid-western Kyushu,
950 Japan. Water depth isopleths every 10 m were made by contouring (Surfer®8; Golden
951 Software) point data provided by the Hydrographic and Oceanographic Department, Japan
952 Coast Guard. Tidal flat areas, including the Tomioka sandflat, are indicated in black. Stn A in
953 Amakusa-nada was regularly visited for water and plankton sampling

954

955 Fig. 2. Newly fertilized embryo-volume-frequency distribution and newly hatched zoea I-total
956 length-frequency distribution of *Nihonotrypaea harmandi* specimens collected from late
957 August to early September 2014. The 2 normal distributions were fitted to each frequency
958 distribution

959

960 Fig. 3. Daily occurrence of decapodids of *Nihonotrypaea harmandi* in the 2 rearing tanks
961 between 3 August (Day 26) and 17 August (Day 40) 2013. Decapodids emerged first on Day
962 26. The 2 normal distributions were fitted to each frequency distribution

963

964 Fig. 4. Shift in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values (solid and blank circle plots, respectively) for the
965 whole-body tissue of *Nihonotrypaea harmandi* zoeae and decapodids in relation to that for
966 food (diatom, *Chaetoceros gracilis*: crosses) during the feeding experiment. See text for
967 sample numbers (n_s). The samples retrieved on Day 40 for stable isotope analysis consisted of
968 zoeae V and VI only. Dotted line and broken line in each panel: mean values for *N. harmandi*
969 and *C. gracilis*, respectively. $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ designate the trophic enrichment factors (TEFs)

970

971 Fig. 5. Shift in range or mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ values for particulate organic matter (POM)
972 with microbial degradation under dark conditions in the laboratory in June and July 2013. See
973 text for sample numbers (n_s)

974

975 Fig. 6. Vertical profiles of (a) abundance and (b) biovolume of nano- and micro-sized plankton
976 and their taxonomic compositions at Stn A in Amakusa-nada off mid-western Kyushu on 7
977 August 2012. Dt: diatom; Df: dinoflagellate; C: ciliate; O: other group including filamentous
978 cyanobacteria and unidentified organisms

979

980 Fig. 7. Dual bulk stable isotope (SI) plots for particulate organic matter (POM), zooplankton,
981 and zoeae I–VI of *Nihonotrypaea harmandi* collected from Stn A in Amakusa-nada off
982 mid-western Kyushu in (a) 2012 and (b) 2013. Samples from multiple cruises during the
983 summer were compiled for each year. Mean values for 2 successive zoeal stages inclusive (I
984 and II, III and IV) or the single mean values for the zoeae V and VI for *N. harmandi* and the
985 mean (±SD) values for the other components are indicated (see text for sample numbers, n_s).
986 Star: mean values for zoeae V and VI inclusive (SDs are omitted for simplicity; see text for
987 values). SI values for zooplankton in 2012 were distinctly lighter for the smaller 2 fractions
988 (0.3–0.8 and 0.8–1.2 mm in size) than for the larger 4 fractions (>1.2 mm), so that the
989 zooplankton were separated into 2 groups at 1.2 mm. The mean SI values of the 2 expected
990 food sources (FSs) corresponding to the mean values of the *N. harmandi* zoeae V and VI
991 inclusive were calculated from respective trophic enrichment factors (TEFs): FS-1 from zoeal
992 whole body-specific TEFs ($\Delta\delta^{13}C$ = 2.0‰; $\Delta\delta^{15}N$ = 1.9‰; Fig. 4) and FS-2 from *N. harmandi*
993 juvenile whole body-specific TEFs, respectively (based on Fig. 5 in Yokoyama et al. 2005).
994 Dark-shaded smaller box (rectangular area) and light-shaded larger box: expected smaller and
995 larger ranges of SIs for the presumed degraded-POM, respectively, derived from the mean SIs
996 for the POM and its SDs, respectively. The positions of 2 opposite corner points on the smaller
997 box are indicated by 2 arrows, each subjected to microbial degradation of the POM at the
998 corresponding daily decreasing rate in its mean SI values for 1 wk (Fig. 6)

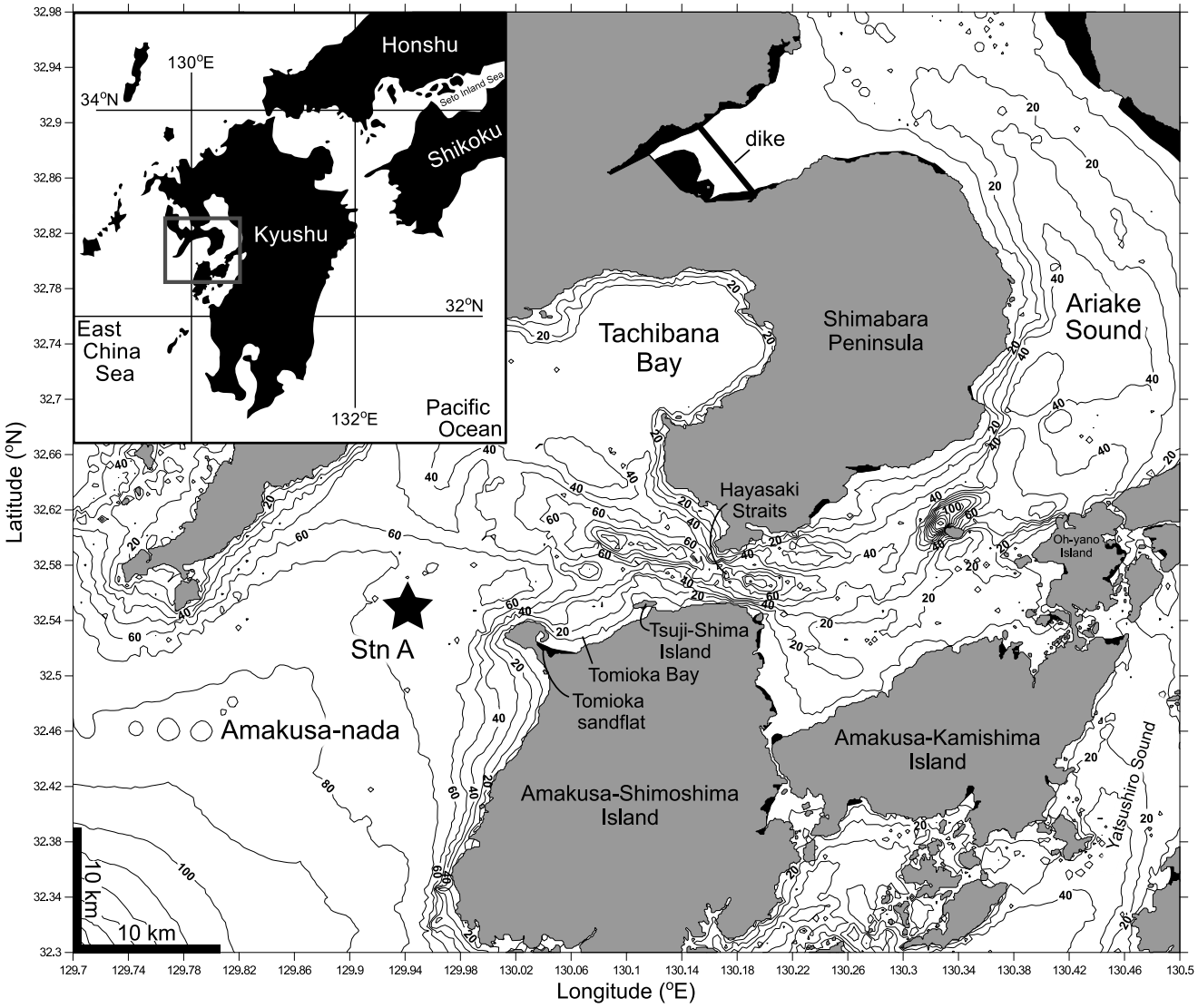

Fig. 1 (Umezawa et al., revised)

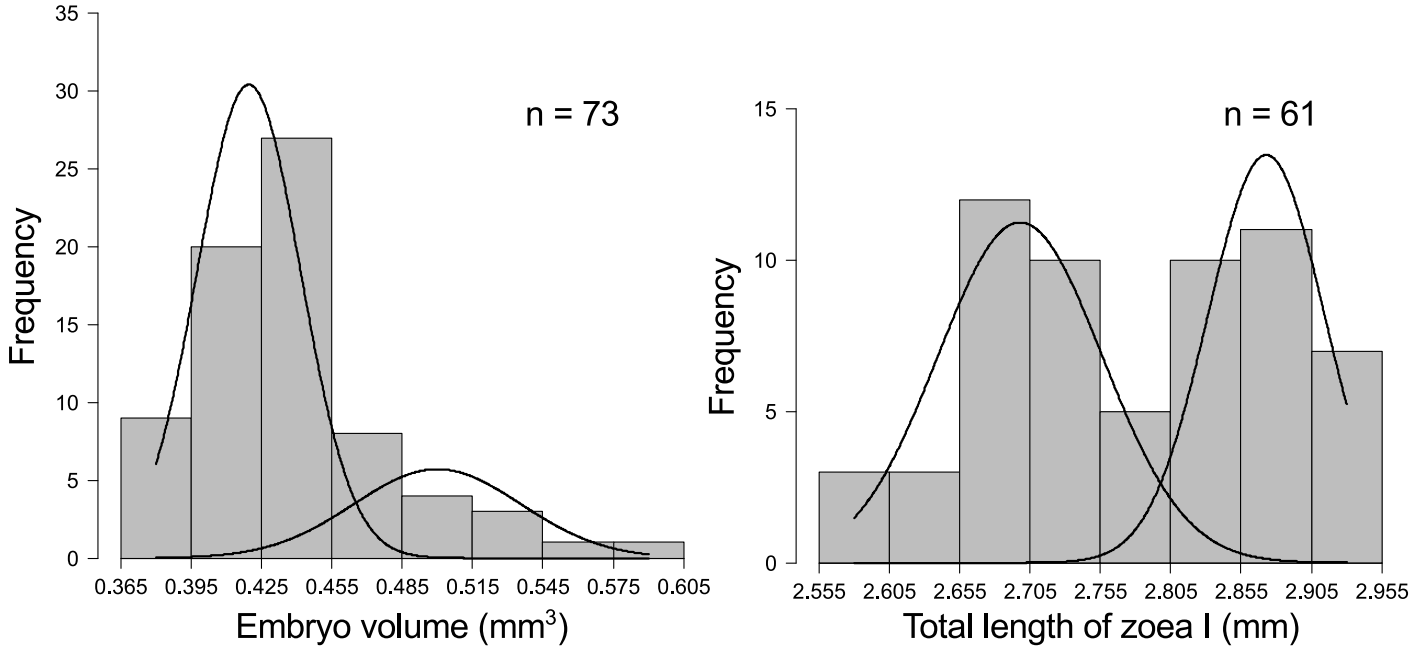

Fig. 2 (Umezawa et al., revised)

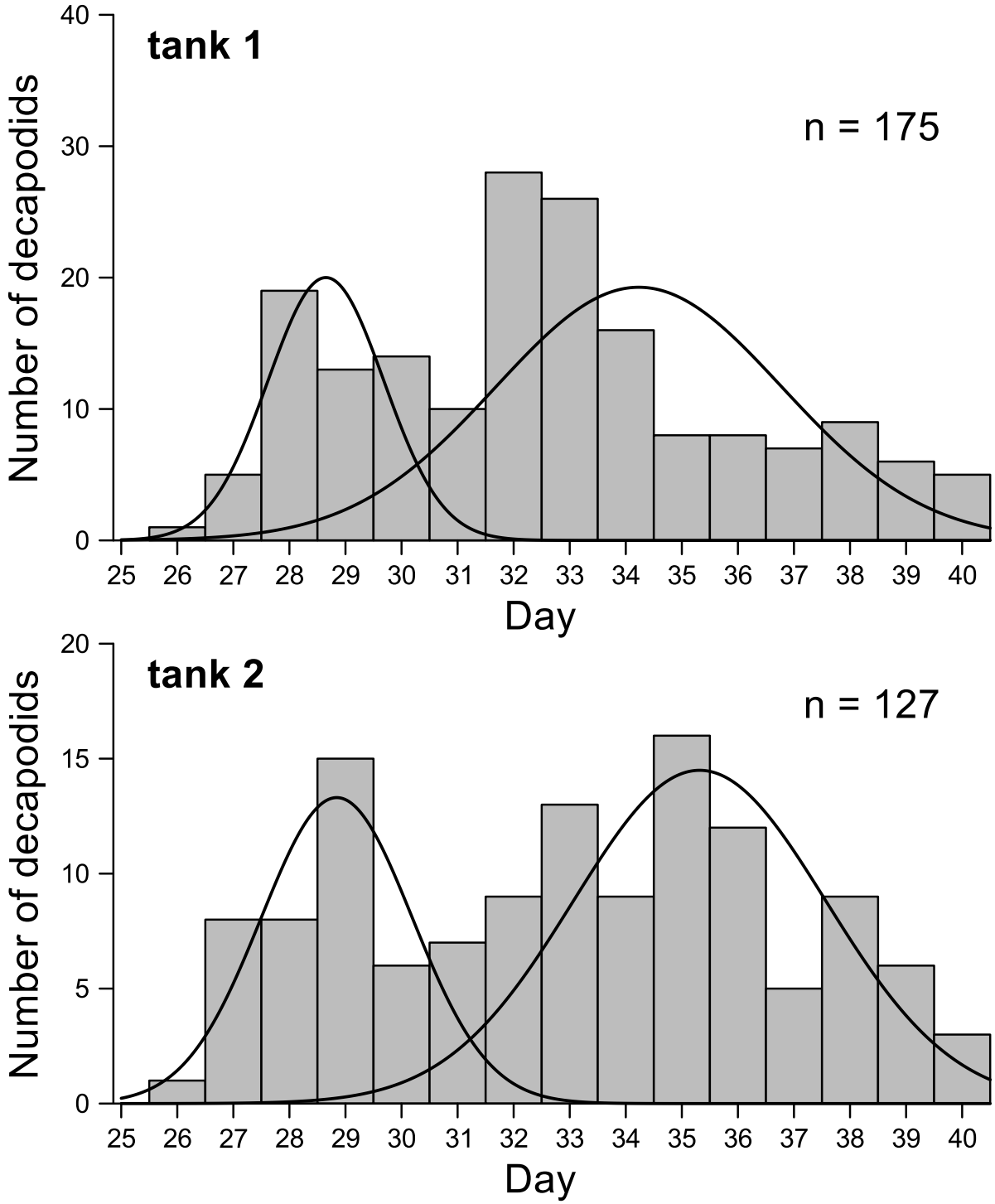

Fig. 3 (Umezawa et al., revised)

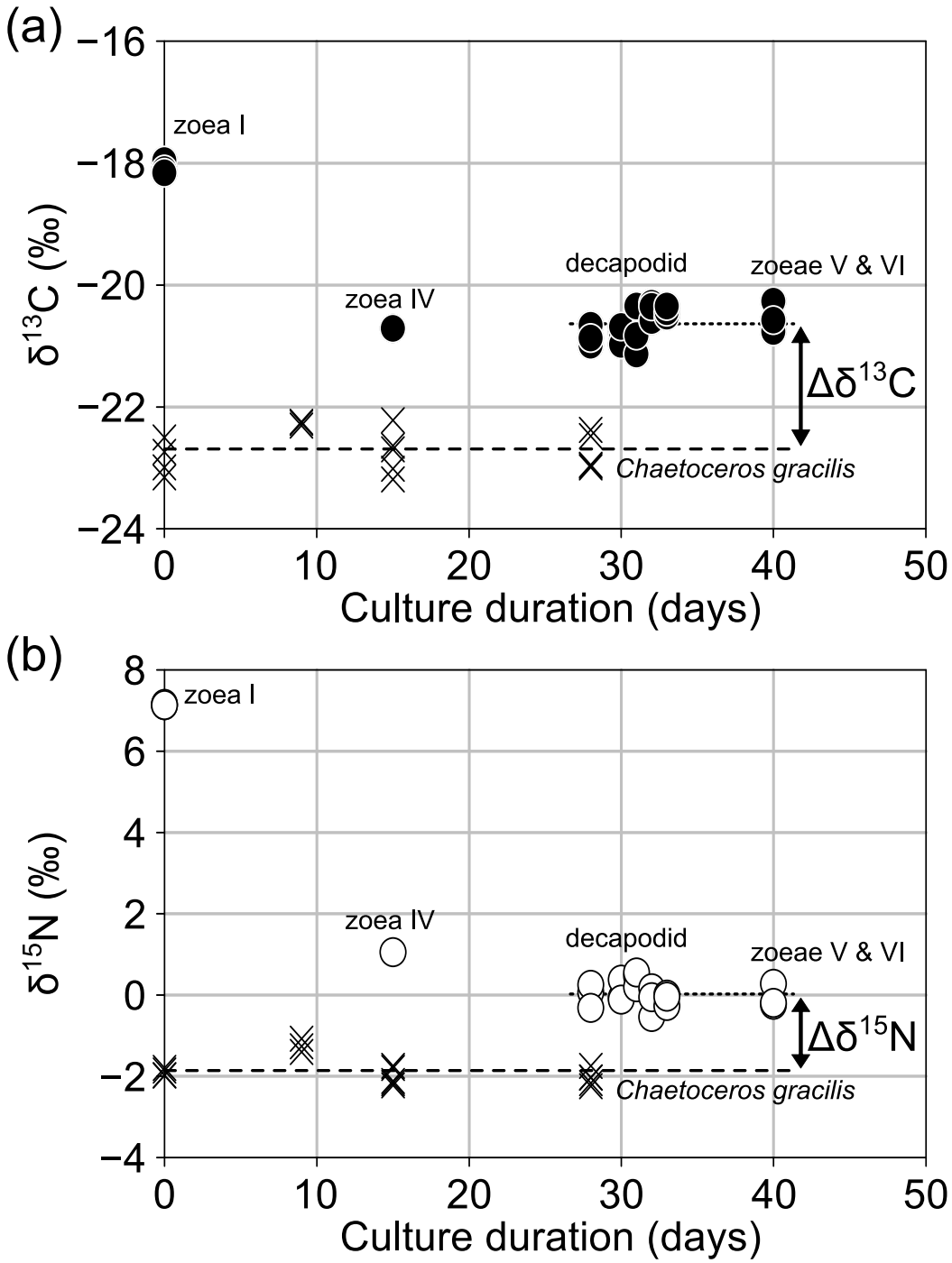

Fig. 4 (Umezawa et al., revised)

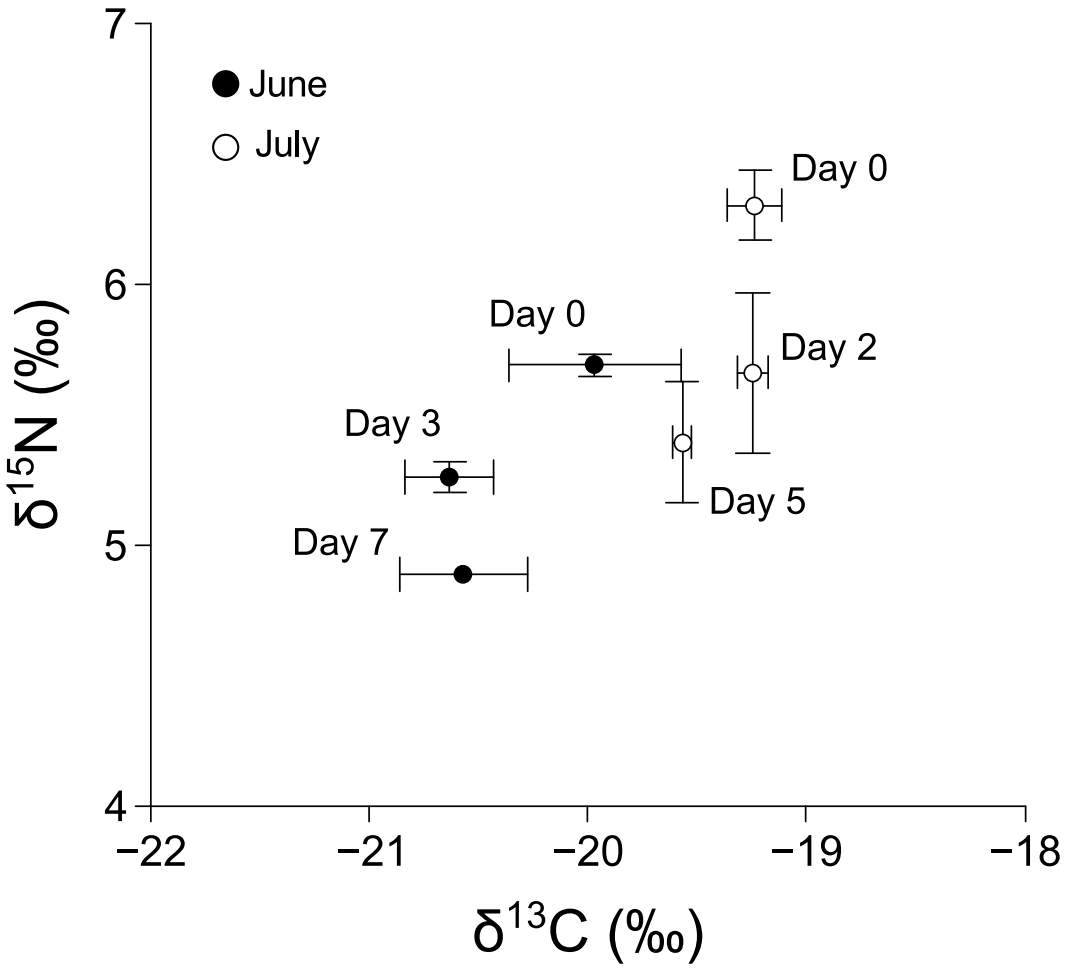

Fig. 5 (Umezawa et al., revised)

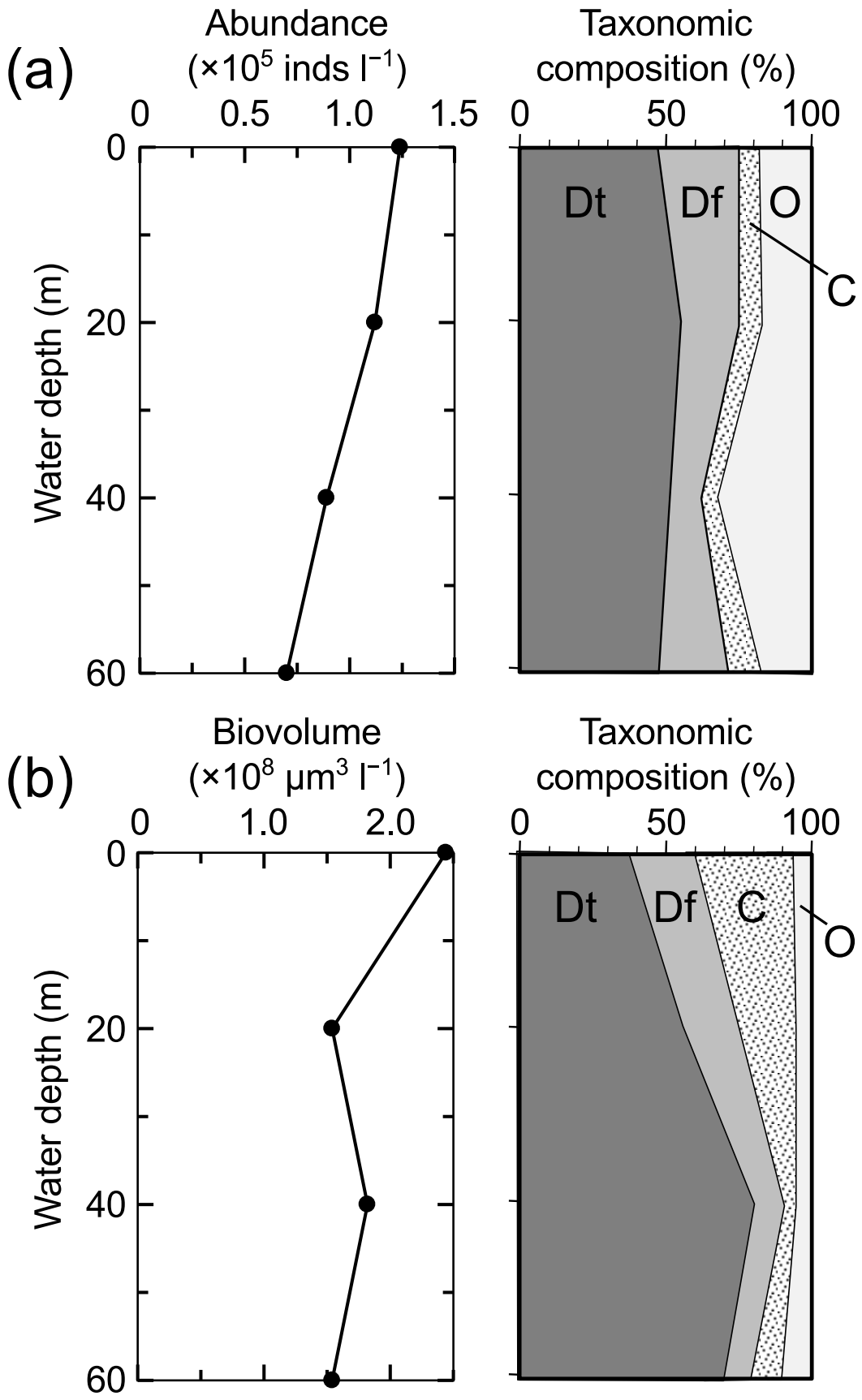

Fig. 6 (Umezawa et al., revised)

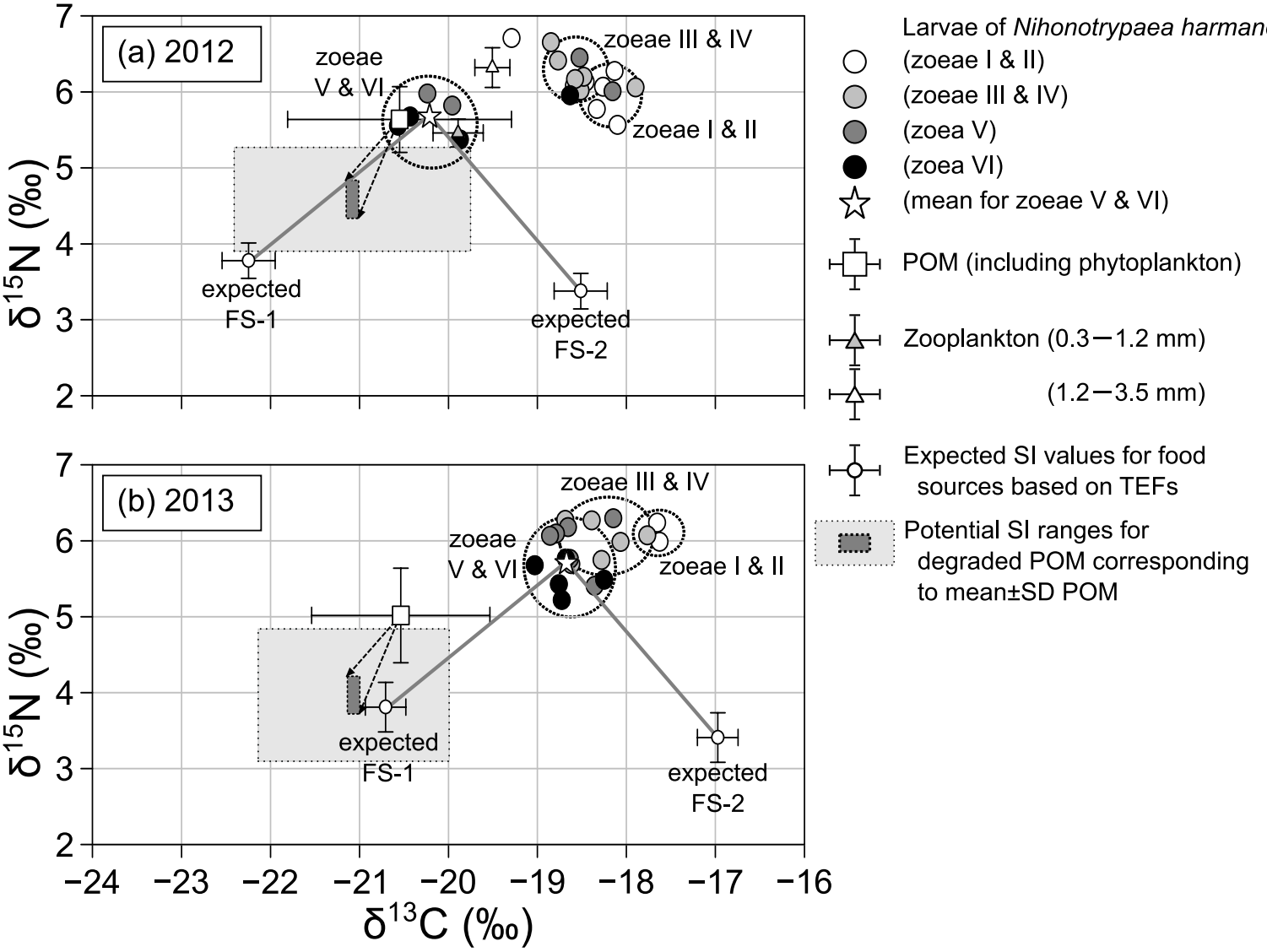

Fig. 7 (Umezawa et al., revised)